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EFFECTS OF SULPHUR DEFICIENCY ON METABOLISM IN TOMATO[1]

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(WITH ONE FIGURE)

Introduction

In connection with studies of protein and carbohydrate metabolism (**26, 27, 28, 29**), and of ammonium and nitrate nutrition (**42**), investigations have been made of effects of deficiency of various essential elements (**29, 30, 31**). This paper records the results of a similar series of experiments on deficiency of sulphur. Most of the experiments were conducted with tomato, although apple, narcissus, and asparagus were also used. This work does not include a study nor a review of the significance of sulphur in soils. An extensive review of the literature on this subject is given by JOFFE (**18**), together with the report of his own investigations. Later work on soil fertilization with sulphur-containing compounds is reported by FRAPS (**15**), who likewise reviews much of the literature.

Chemical methods

PLANT FRACTIONS.—The stem tip or upper 25 mm. of stem tissue was in some experiments removed and analyzed separately. The remainder of the stem was divided equally according to length into the two fractions which are termed *lower stem* and *upper stem*. The *petiole* fraction includes all vein and petiole tissue to which the mesophyll of the blade is not directly attached; the smaller veins are included with the other tissues

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The Kjeldahl, mineral, and sulphur determinations were made in the laboratory of C. S. CATHCART, for whose cooperation the writers wish to express their appreciation.

of the *blades* fraction, and have been estimated (**29**) to constitute about 35 to 50 per cent. by volume of that fraction. The analytical sample termed *roots* consists principally of fibrous roots and does not include the small taproot or the region that is intermediate in structure between the root and the stem.

The analytical plant fraction of paper-white narcissus which will be referred to as *centers* consists of the unexpanded floral organs and true leaves. Any top growth which was present is included with the centers for analysis and will be termed *centers* and *tops*. The storage scales, together with the disk-like stem of the bulb, are designated *storage tissue*. The entire root system is included in the analytical fraction *roots*. Details concerning the technique employed in separating these bulb fractions are given in another report (**28**).

NITROGEN FRACTIONS.—Fresh tissue only was employed for nitrogenous fractionation. The manner of extraction with water according to the method of CHIBNALL (**5**) has already been described (**29**). The term *protein nitrogen* is used to designate a group or groups of the more complex nitrogenous constituents, and includes both unextracted nitrogen and extracted coagulable nitrogen. The fraction termed by some investigators *total water-soluble nitrogen* is synonymous with *total extracted nitrogen*. In this publication no report is given of this fraction, as such a determination is chiefly indicative of the degree of grinding of the plant tissue, and is of doubtful metabolic significance (**28**). *Soluble nitrogen* as used in this work refers only to that fraction of extracted nitrogen which has been freed of coagulable nitrogen after being boiled and dilute acetic acid added (**5**).

Ammonia was estimated by the method of VAN SLYKE (**43**) on aliquots of the soluble-nitrogen fraction.

Amide nitrogen was determined by the usual procedure on aliquots of soluble nitrogen. It should be mentioned that for the determination of amide nitrogen sulphuric acid has always been employed in these laboratories, because with hydrochloric acid consistent results have not been obtained. Both CHIBNALL (**6**) and VICKERY (**44**) have recently reported that in the presence of nitrates, hydrochloric acid may not be employed in the determination of amide nitrogen, but that sulphuric acid is satisfactory. The humin nitrogen formed was found to be negligible in amount and the filtrate was employed for the estimation of basic nitrogen by precipitation with phosphotungstic acid (**29**).

Alpha amino nitrogen was estimated by the VAN SLYKE method on aliquots of the phosphotungstic acid filtrate (**29**).

Nitrate nitrogen was determined according to the procedure of SESSIONS and SHIVE (**37**) on aliquots of ammonia-free soluble nitrogen.

Other nitrogen was calculated as the difference between soluble nitrogen and the sum of the soluble-nitrogen fractions.

SULPHUR FRACTIONS.—Preliminary trials showed only a slight loss of volatile sulphur from freshly minced stem and blades of tomato that were dried in a current of air at 80° C. The amount lost was too small for quantitative estimation by PETERSON's method (**32**), whereas cabbage leaves subjected to the same treatment yielded a considerable quantity of volatile sulphur. Consequently the determination of total sulphur in tomato tissue was carried out according to Official Methods (**2**), with minced tissue that had been dried rapidly at 80° C. in a current of air and had then been ground as previously described (**28**). For estimation of sulphur fractions, fresh tissue only was employed, because trials showed that the method of drying just described caused a marked decrease in proteins and a corresponding increase in sulphate-free soluble sulphur. The percentage of sulphates was found to be the same in dried as in fresh tissue. However, only in case of narcissus (tables X and XI) was dried material used for sulphate determinations. Aliquots of the protein or coagulum-free extract obtained as described under *nitrogen fractions* were used and the Official (**2**) procedure followed for determination of *sulphates*[2] and total *soluble sulphur*. *Protein sulphur* was computed as the difference between total sulphur and soluble sulphur.

Aliquots of the phosphotungstic acid precipitate obtained from the coagulum-free extract were analyzed for cystine according to the method of PLIMMER (**33**), but the quantity of cystine was insufficient for macrochemical estimation even in large aliquots of tomato stem or blade tissue. The extract had previously been subjected only to the mild acid hydrolysis and sodium carbonate treatment of the amide determination. Analyses for cystine and cysteine were also made by the colorimetric method of SULLIVAN (**38, 39, 40**); pigments present in the tomato-plant tissue interfered with quantitative determinations, although the results clearly indicated that traces of cysteine and cystine were present in the protein-free extract of whole tomato stems.

An attempt was made to adapt the colorimetric nitro-prusside reaction to a quantitative estimation of total S-H sulphur present in the protein-free extract of tomato-plant tissue; but the slight color which occurred was fleeting, and plant pigments interfered.

Microchemical methods

Microchemical tests were made on sections of plant tissue as recom-

[2] Any sulphites present are included in the sulphate determination. Nitrates at concentrations usually found in tomato were added to nitrate-free tomato stem extract. Recovery of sulphate was not apparently influenced by the added nitrate.

mended by ECKERSON (**14**). Some of these tests for sulphur-containing compounds are given later.

SULPHATES.—One per cent. benzidine in 3 per cent. hydrochloric acid was employed in testing for sulphates. It was the only reaction that was found satisfactory or sufficiently sensitive for testing for sulphates in plant tissue. This test in the presence of sulphates gave glistening white scale-like crystals of benzidine sulphate. Other reactions (**4**) were found more or less satisfactory in testing for sulphates in drops of expressed juice.

SULPHITES.—Extensive trials of many different reagents resulted in only one satisfactory method for testing for sulphites in plant tissue. To thin sections of plant tissue was added a drop or two of 10 per cent. sodium tetrathionate, followed in a few minutes by a drop of 10 per cent. barium chloride. Any sulphites present even in extreme dilution reacted at once to form barium thiosulphate crystals, which look much like starch grains but have a higher refractive index. The crystals often appear in pairs like two mushrooms, with a heavy black border, which is caused by light refraction. This test for sulphites is based upon the fact that salts of polythionic acid react with sulphites to form thiosulphates (**34**), which in turn react with barium chloride to form characteristic barium thiosulphate crystals.

GLUTATHIONE, CYSTEINE.—The nitro-prusside reaction as employed by KOZLOWSKI (**19**) and WHITE (**48**) was used as a test for total S-H or reduced sulphur. It is not, however, specific for either glutathione (**38, 39, 40**) or cysteine, although it has frequently been used by investigators as an index of the glutathione content of plants. Many studies arbitrarily reported as on glutathione are more strictly speaking investigations of the S-H sulphur content of plants. The colorimetric method of SULLIVAN (**38, 39, 40**) was used in making qualitative tests for cysteine on sections of plant tissue, on expressed juice, and on aliquots of protein-free extract of plant tissue.

CYSTINE.—Microchemically cystine was easily identified by its characteristic crystal form, comparative insolubility in water, high refractive index, and chemical reactions.

Experimental methods

Tomato plants (*Lycopersicon esculentum* Mill.) of the variety Marglobe were grown in sifted loam soil in individual 4-inch pots. On May 12, 1931, at the time of commencing experimental treatments, there were available 2500 plants. On that date, 1200 plants were selected for uniformity; the roots of each plant were washed free of soil and the two lower leaves were removed. Two hundred plants were used for initial analysis and 1000

plants were transplanted for experimental treatments to washed quartz sand in new 10-inch clay pots, one or two plants to a pot. The pots were set in shallow enamelware pans. For the period of these experiments no difference in growth was observable between plants that were grown one and those grown two in a pot.

Immediately after being transplanted from soil to sand culture, all of the plants were subjected to nutrient treatments.[3] Some of them received the complete or plus-sulphur, others the minus-sulphur, and a third group the minus-nitrogen solution, as indicated in table I. Also from time to time some of the plants were shifted from one nutrient treatment to another. Each pot received two liters of solution daily, and twice a week each culture was thoroughly flushed with distilled water, after which fresh nutrient solution was applied.

Sufficient iron for subsequent growth was present in the initial plants or possibly as impurities in the salts employed. Likewise, perhaps for the same reason, it was not found necessary to apply boron or manganese; at least the complete-nutrient plants which were allowed to mature grew luxuriantly and produced a heavy crop of fruit. Further, no apparent effect was produced on the minus-sulphur or minus-nitrogen or complete-nutrient plants by the addition of boron or manganese at the rate of one quarter part per million of each.

The plants were grown in a greenhouse at New Brunswick, during the spring and early summer of 1931, under conditions of temperature and humidity suitable for the commercial production of tomatoes. The plants were grown under the seasonal light conditions of the greenhouse, with the exception of some that were subjected to a period of continuous darkness at a practically constant temperature of 20° C. Each plant was kept pruned to a single stem, and blossoms were hand-pollinated daily; but pollen from flowers of one lot of plants was not used for pollination of flowers of another group. Thirty or more plants of a series were harvested for analysis at 7 A. M. on the several dates indicated in the tables of analytical data.

Results

On May 12, at the time the respective nutrient treatments (table I) were started, the initial tomato plants were about 30 cm. in height, dark

[3] The rather low (29) concentration of nitrate in the nutrient solutions (table I) was purposely maintained in order that there might be no accumulation of nitrates in the tissues of plants which were active in amino acid synthesis. A relatively high percentage of nitrate in a given lot of plants should then be of considerable interpretive value. If tomato plants receive a high external nitrate supply they become practically saturated with nitrate (29), even though they are rapidly synthesizing organic nitrogenous compounds.

green and somewhat soft or succulent; cell walls of stem fibers were little thicker than walls of adjacent parenchymatous tissues; and xylem elements were still comparatively thin-walled. The plants were very low in sugars. The stems contained only 6.10 per cent. dry matter (table II) and were practically devoid of starch except in the endodermis; on the other hand, they were high in organic nitrogen, high in organic sulphur, and contained considerable sulphate and nitrate (tables II and III). Traces of sulphite were also present, particularly in the phloem region of the stem.

TABLE I

PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED*

	SOLUTION				
	K_2SO_4	$Ca(NO_3)_2$	$Mg(H_4PO_4)_2$	$CaCl_2$	KCl
Plus-S	0.0045	0.0090	0.0045	.....	.....
Minus-S	.....	0.0090	0.0045	.....	0.0045
Minus-N	0.0045	.....	0.0045	0.0090	.....

* Each solution has a total osmotic concentration value of 1.25 atmospheres.

Attention is also called to the fact that these very actively vegetative plants were high in sulphur. Tests of this protein-free aqueous extract showed that the extract contained only a trace of S-H sulphur (nitro-prusside reaction) (**18, 48**). However, rather intense reactions for total S-H sulphur were obtained, but only in the cambium region and in other meristematic tissues, namely, stem and root tips. SULLIVAN'S (**38, 39, 40**) reaction apparently indicated a faint trace of cysteine, likewise limited exclusively to meristematic tissue and not sufficient in quantity to be detected in the extract from whole stems. In view of the fact that according to the nitro-prusside reaction there was apparently much more total reduced sulphur than could be accounted for by the presence of cysteine (SULLIVAN'S method), it would seem not improbable that glutathione was mainly responsible for the positive S-H reaction of active tissues of the plant.

Under the microscope there was observed an occasional naturally occurring crystal of cystine in the comparatively alkaline tissue of the phloem (**9**). The protein-free aqueous extract did not contain a measurable quantity of cystine, as none could be detected by the method of SULLIVAN or by the procedure of PLIMMER (**33**).

PLANTS WHICH RECEIVED COMPLETE NUTRIENT SOLUTION

During the period of these experiments (May 12–July 8), the plus-sulphur or complete-nutrient plants (fig. 1) were moderately vegetative.

TABLE II

CONTINUOUSLY PLUS-SULPHUR AND CONTINUOUSLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT						
	MAY 12	JUNE 9		JUNE 19		JULY 8	
	INITIAL PLANTS	PLUS-S	MINUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Total sulphate-free S	0.328	0.136	0.112	0.162	0.138	0.166	0.120
Protein S	0.247	0.136	0.091	0.099	0.022	0.166	0.009
Sulphate-free soluble S	0.081	Trace	0.021	0.063	0.116	Trace	0.111
Sulphate S	0.127	0.111	0.011	0.124	Trace	0.173	Trace
Total S	0.455	0.247	0.123	0.286	0.138	0.339	0.120
Total nitrate-free N	2.297	1.315	1.365	1.054	1.255	1.007	1.383
Protein N	1.133	0.915	0.980	0.735	0.709	0.770	0.691
Nitrate-free soluble N	1.164	0.400	0.385	0.319	0.546	0.237	0.692
Basic N	0.280	0.173	0.093	0.071	0.080	0.052	0.110
Amino N	0.561	0.147	0.140	0.137	0.200	0.074	0.276
Amide N	0.272	0.030	0.086	0.049	0.127	0.038	0.158
Ammonia N	0.047	0.020	0.062	0.040	0.100	0.031	0.101
Other N	0.004	0.030	0.004	0.022	0.039	0.042	0.047
Nitrate N	0.279	0.080	0.150	0.060	0.183	0.065	0.301
Total N	2.576	1.395	1.515	1.114	1.438	1.072	1.684
Dry matter	6.100	13.000	14.150	15.250	17.400	18.800	20.350

TABLE III

CONTINUOUSLY PLUS-SULPHUR AND CONTINUOUSLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT						
	MAY 12	JUNE 9		JUNE 19		JULY 8	
	INITIAL PLANTS	PLUS-S	MINUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Total sulphate-free S	0.020	0.018	0.016	0.025	0.024	0.031	0.024
Protein S	0.015	0.018	0.013	0.015	0.004	0.031	0.002
Sulphate-free soluble S	0.005	Trace	0.003	0.010	0.020	Trace	0.022
Sulphate S	0.008	0.014	0.002	0.019	Trace	0.033	Trace
Total S	0.028	0.032	0.018	0.044	0.024	0.064	0.024
Total nitrate-free N	0.140	0.171	0.193	0.161	0.218	0.189	0.281
Protein N	0.069	0.119	0.139	0.112	0.123	0.144	0.140
Nitrate-free soluble N	0.071	0.052	0.054	0.049	0.095	0.045	0.141
Basic N	0.017	0.022	0.013	0.010	0.014	0.010	0.022
Amino N	0.034	0.019	0.020	0.021	0.035	0.014	0.056
Amide N	0.017	0.004	0.012	0.007	0.022	0.007	0.032
Ammonia N	0.003	0.003	0.009	0.006	0.017	0.006	0.021
Other N	0.001	0.004	0.002	0.005	0.007	0.008	0.010
Nitrate N	0.017	0.010	0.021	0.009	0.032	0.013	0.061
Total N	0.157	0.181	0.214	0.170	0.250	0.202	0.342

The initial plants contained practically no starch, and it was not until June 9 that there began to occur considerable starch storage. At about that time many fruits were set. Although the plants were not extremely active nor soft, they were nevertheless dark green and did not lose any of their lower leaves during the period of the experiments. The rate of growth, however, was much slower than that of less fruitful plants that

FIG. 1. Tomato plants, June 23, 1931: from left to right, plus-sulphur and minus-sulphur. Compare relative diameter of stems of the two plants. Note that sulphur-deficient plant is so stiff and woody that no stake is required to support it; that it is lighter in color; and that a single fruit has developed whereas fruits have not yet set on the plus-sulphur plant.

received a complete nutrient solution with three times the concentration of nitrates.

On July 8, stems of the fruitful, moderately vegetative plants that were supplied with the complete nutrient solution (table I) contained the

following percentage of carbohydrates on a dry weight basis: reducing sugars 4.20, sucrose 4.35, and starch and dextrin 12.17. The moderately low concentration of nitrogenous constituents is indicated in tables II and III.

The percentage of organic sulphur and sulphates was about half that found in the initial plants. Further, most of the sulphate-free sulphur was in the form of protein (tables II and III). Microchemical tests also indicated that there was much less S-H sulphur than in the initial plants. Strong, positive reactions for reduced sulphur, however, were obtained in the vicinity of stem and root tips and in the cambium region, especially of the upper half of the stem.

Sulphur-deficient plants

Symptoms of sulphur deficiency developed very gradually, but by June 9 the upper half of the stem of the minus-sulphur plants was very small in diameter and produced no axillary shoots. The total stem length, however, was equal to or greater[4] than that of the plants supplied with the complete nutrient solution (fig. 1). The lower leaves especially, and by July 8 even the upper leaves, were yellowish green with purple veins. The newly formed leaflets were of small area and were spaced far apart on the rachis. Stems were stiff and woody, and the few fruits formed were set and matured early (fig. 1). The minus-sulphur plants looked as if they had been gradually but not completely limited as to their nitrogen supply. This appearance was borne out by the root systems, which had the general character of those usually produced by low-nitrogen plants (**20, 26, 29**). The roots were somewhat extensive, but of small diameter because there was practically no cambium nor secondary thickening. Cells did not show any deformity nor injury, however, as in calcium deficiency (**31**).

Macrochemical analyses for essential mineral elements showed that sulphur deficiency did not materially affect the concentration of most of the mineral elements. The concentration of calcium was somewhat high, however, caused apparently by heavy deposits of calcium oxalate, especially in the phloem. The phloem of the minus-sulphur plants was about pH 5.6 as compared with 7.0 in the complete-nutrient series. Other mineral deficiencies have been shown (**10, 30**) to result in plant tissue of comparatively low pH and high concentration of calcium oxalate. There was no accumulation of any other mineral element; in fact, computed on a dry-

[4] The limit of ability of the sulphur-deficient plants to continue stem elongation was not determined. A few specimens were retained until August 1, and even at that time stems were still increasing in length, although the last-formed 30 cm. of stem was not more than 2 mm. in diameter.

weight basis, analyses of July 8 show that the percentage of potassium in the stems of the plus-sulphur plants was 2.98 as compared with 1.99 in the sulphur-deficient series. The lower figure, however, represents a concentration of potassium that is more than adequate for good growth of tomato (**30**). The lower concentration is probably associated with the fact that the minus-sulphur plants had little meristematic tissue. Active tissue is notably high in potassium (**30**).

When analyzed on July 8, the stems of the minus-sulphur plants contained the following percentage of carbohydrates computed on a dry weight basis: reducing sugars 6.32, sucrose 7.00, and starch and dextrin 14.79. The anatomical structure also corresponded closely to that of typical nitrogen-deficient high-carbohydrate plants (**20, 30**). The stems had no active cambium except in and near the stem tip; the walls of both internal and external fibers were extremely thick; and there was a very high proportion of thick-walled xylem and collenchyma tissue.

Nitrates were not low; in fact, when the last harvest was made (tables II and III) the stems of the sulphur-deficient lot contained nearly six times the concentration of nitrate found in the complete-nutrient plants. Total organic nitrogen also was consistently a little higher in the minus-sulphur group; this was caused mainly by accumulation of basic, amino, and amide nitrogen. Ammonia also was high (tables II and III). The percentage of total organic sulphur was not materially different in the two groups of plants, but in the plus-sulphur series nearly all of it was protein; whereas in the plants lacking an external sulphate supply there was little protein sulphur but much soluble sulphate-free sulphur. This non-protein water-soluble organic sulphur gave negative tests for cysteine, cystine, and glutathione. Its form was not determined. The extremely small stem tip of the sulphur-deficient plants appeared, however, to give a very faint S-H reaction (nitro-prusside test).

The concentration of sulphur in different parts of the plant is indicated in tables IV and V.

Sulphur-deficient plants shifted to plus-sulphur nutrient treatment

A few seconds after sulphur-deficient plants were supplied with the complete nutrient solution, sulphates were observed adsorbed on the outer pectic layers of the root hairs, and almost immediately could be detected within them. Five minutes later sulphates appeared in the fine roots, and after six hours large quantities of sulphate were found in the base of the stem and smaller quantities in the remainder of the stem and in the leaves.

The appearance of sulphites in different parts of the plants was almost simultaneous with that of sulphates, although the maximum concentration

TABLE IV

CONTINUALLY PLUS-SULPHUR AND CONTINUALLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

PLANTS HARVESTED FOR ANALYSIS JUNE 30, 1931

MATERIAL	TREATMENT						
	TOTAL SULPHATE-FREE S		SULPHATE S‡	TOTAL S		DRY MATTER	
	PLUS-S	MINUS-S	PLUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Tip of stem	0.227	0.131	0.189	0.416	0.131	7.89	17.26
Upper stem	0.152	0.111	0.131	0.283	0.111	17.10	19.00
Lower stem	0.119	0.125	0.096	0.215	0.125	20.50	21.70
Fibrous roots*	0.318	0.238	0.463	0.781	0.238	9.02	10.20
Upper petioles	0.221	0.020	0.279	0.500	0.020	11.95	10.32
Lower petioles	0.177	0.103	0.485	0.662	0.103	11.80	11.20
Upper blades	0.387	0.152	0.747	1.134	0.152	17.00	16.80
Lower blades	0.261	0.214	1.871	2.132	0.214	15.50	17.75
Flesh of ripe fruit	0.149	0.106	0.063	0.212	0.106	6.92	5.94
Seed of ripe fruit†	0.228	0.141	0.021	0.249	0.141	.....	.....

* Computed on ash-free basis.

† Computed as percentage of air-dry weight of seeds.

‡ Minus-S plants did not contain sufficient sulphate to be detected by macrochemical analysis (2).

TABLE V

CONTINUALLY PLUS-SULPHUR AND CONTINUALLY MINUS-SULPHUR TOMATO PLANTS
SULPHUR FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER
PLANTS HARVESTED FOR ANALYSIS JUNE 30, 1931

MATERIAL	TREATMENT				
	TOTAL SULPHATE-FREE S		SULPHATE S‡	TOTAL S	
	PLUS-S	MINUS-S	PLUS-S	PLUS-S	MINUS-S
Tip of stem	0.018	0.022	0.015	0.033	0.022
Upper stem	0.026	0.021	0.022	0.048	0.021
Lower stem	0.024	0.027	0.020	0.044	0.027
Fibrous roots*	0.029	0.024	0.041	0.070	0.024
Upper petioles	0.026	0.002	0.034	0.060	0.002
Lower petioles	0.021	0.012	0.057	0.078	0.012
Upper blades	0.066	0.026	0.066	0.193	0.026
Lower blades	0.040	0.038	0.290	0.330	0.038
Flesh of ripe fruit	0.010	0.006	0.005	0.015	0.006
Seed of ripe fruit†	0.228	0.141	0.021	0.249	0.141

* Computed on ash-free basis.

† Computed as percentage of air-dry weight of seeds.

‡ Minus-S plants did not contain sufficient sulphate to be detected by macrochemical analysis (2).

of sulphite was not reached until about 24 hours following the shift to plus-sulphur treatment.

Six hours after absorption of sulphate the roots gave faint but positive reactions for S-H sulphur; somewhat later much stronger S-H reactions occurred, particularly in the cambium and phloem regions and in all tissues of the tops that were not entirely mature. Some, but only a small part, of the reduced sulphur was cystein (SULLIVAN reaction); and therefore by inference (page 567) it may be suggested that most of the reduced sulphur was glutathione.

Seventy-two hours after the shift to plus-sulphur treatment (June 23, tables VI and VII) there was found a decrease in nitrate and an increase in organic nitrogen. Accompanying the decrease in nitrate, strong reactions for nitrite were obtained in the then more alkaline phloem region of roots and tops. Tables VI and VII also show that following the shift to complete nutrient there occurred a marked and very rapid increase in percentage of protein sulphur. Total organic sulphur showed no increase on a percentage basis. This is probably because the plants increased rapidly in volume immediately following the shift. On an absolute amount basis there was unquestionably an increase in total organic sulphur.

The response to an application of sulphates was extremely rapid. The plants had turned much darker green within twelve hours. Even the lower yellow leaves turned green during that time, and by the end of the second and third day there was a marked increase in diameter of newly formed stem and in area of newly developing leaves.

TABLE VI

MINUS-SULPHUR TOMATO PLANTS SHIFTED TO PLUS-SULPHUR TREATMENT
SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT		
	JUNE 19	JUNE 23	JUNE 30
	MINUS-S	MINUS-S TO PLUS-S*	MINUS-S TO PLUS-S*
Total sulphate-free S	0.138	0.093	0.121
Protein S	0.022	0.052	0.097
Sulphate-free soluble S	0.116	0.041	0.024
Sulphate S	Trace	0.017	0.025
Total S	0.138	0.110	0.146
Total nitrate-free N	1.255	2.253	1.066
Protein N	0.709	1.667	0.617
Nitrate-free soluble N	0.546	0.586	0.449
Basic N	0.080	0.111	0.097
Amino N	0.200	0.273	0.200
Amide N	0.127	0.130	0.082
Ammonia N	0.100	0.082	0.033
Other N	0.039	−0.010	0.037
Nitrate N	0.183	0.077	Trace
Total N	1.438	2.330	1.066
Dry matter	17.400	16.900	15.520

* After June 19, some of the minus-S plants were subjected to plus-S treatment.

TREATMENT IN DARKNESS

The plants which received the nutrient solution lacking nitrogen (minus-nitrogen plus-sulphur) were typical yellow, stunted, woody plants, very low in nitrogen and high in carbohydrates. After twelve days of continuous darkness there was as usual (**26, 29**) a marked drop in carbohydrates, a decrease in protein and a corresponding increase in amino and amide nitrogen (tables VIII and IX). Total nitrogen decreased in percentage because the plants increased greatly in volume and green weight, although there was no external nitrogen supply. The stems showed an

increase in length of 15 to 20 cm., and many new leaves expanded during the interval of twelve days without light. The temperature was about 20° C. In the nitrogen-deficient plants during the darkness period, catabolism seems also to have been the predominant phase, not only of nitrogen but also of sulphur metabolism (tables VIII and IX).

It may further be noted that the minus-nitrogen plants did not accumulate large quantities of sulphate, even though before the shift to

TABLE VII

MINUS-SULPHUR TOMATO PLANTS SHIFTED TO PLUS-SULPHUR TREATMENT
SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT		
	JUNE 19	JUNE 23	JUNE 30
	MINUS-S	MINUS-S TO PLUS-S*	MINUS-S TO PLUS-S*
Total sulphate-free S	0.024	0.016	0.019
Protein S	0.004	0.009	0.015
Sulphate-free soluble S	0.020	0.007	0.004
Sulphate S	Trace	0.003	0.004
Total S	0.024	0.019	0.023
Total nitrate-free N	0.218	0.381	0.165
Protein N	0.123	0.282	0.096
Nitrate-free soluble N	0.095	0.099	0.069
Basic N	0.014	0.019	0.015
Amino N	0.035	0.046	0.031
Amide N	0.022	0.022	0.013
Ammonia N	0.017	0.014	0.005
Other N	0.007	–0.002	0.005
Nitrate N	0.032	0.013	Trace
Total N	0.250	0.394	0.165

* After June 19, part of the minus-S plants were subjected to plus-S treatment.

darkness the sulphate-free soluble sulphur fraction was high (tables VIII and IX) as compared with that of the complete-nutrient plants (tables II and III). This fraction appeared to contain a trace of S-H sulphur but no cystine. Following the period of darkness, newly developed tissue of the minus-nitrogen plants gave apparently more intense S-H reactions.

The treatment in darkness of sulphur-deficient plants resulted in etiolation of newly expanded leaves but did not appear to affect in slightest degree the rate of stem elongation nor any other noticeable phase of growth

as compared with other minus-sulphur plants which were subjected to the seasonal light conditions of the greenhouse during the same period. Carbohydrates decreased materially in the minus-sulphur plants during the 12-day period without light, and there was some indication of catabolism of nitrogenous materials (tables VIII and IX). The same tables of data, however, indicate no material change in percentage nor quality of sulphur fractions.

TABLE VIII

MINUS-NITROGEN AND MINUS-SULPHUR TOMATO PLANTS SHIFTED FROM DAYLIGHT TO CONTINUOUS DARKNESS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND DATE			
	MINUS-N (PLUS-S)		MINUS-S (PLUS-NO_3)	
	JUNE 6	JUNE 18	JUNE 19	JULY 2
	NORMAL LIGHT	PART OF JUNE 6 PLANTS AFTER 12 DAYS' DARKNESS	NORMAL LIGHT	PART OF JUNE 19 PLANTS AFTER 12 DAYS' DARKNESS
Total sulphate-free S	0.154	0.118	0.138	0.154
Protein S	0.117	0.068	0.022	0.035
Sulphate-free soluble S	0.037	0.050	0.116	0.119
Sulphate S	0.071	0.138	Trace	Trace
Total S	0.225	0.256	0.138	0.154
Total nitrate-free N	0.828	0.853	1.255	1.683
Protein N	0.657	0.531	0.709	0.820
Nitrate-free soluble N	0.171	0.322	0.546	0.863
Basic N	0.053	0.060	0.080	0.174
Amino N	0.082	0.164	0.200	0.395
Amide N	0.029	0.107	0.127	0.120
Ammonia N	None	Trace	0.100	0.107
Other N	0.007	−0.009	0.039	0.067
Nitrate N	None	None	0.183	0.257
Total N	0.828	0.853	1.438	1.940
Dry matter	15.550	12.740	17.400	12.530

Certain other experiments are considered in the discussion that follows. Results of cooperative studies with Dr. ECKERSON on sulphate and nitrate reduction are also included.

Discussion

EXTERNAL RESPONSES.—It has been found (**31**) that if seedling tomato plants are transplanted to sand cultures deficient respectively in nitrogen

(**20, 29**), phosphorus (**10**), or potassium (**30**), the effects of nitrogen or phosphorus deficiency are evident in a comparatively short time, but conspicuous symptoms of lack of potassium (**30**) are not usually apparent until much later. Although symptoms of lack of these respective elements may not occur simultaneously, the effects upon the general appearance of the plants are similar. The lower leaves and lower stem are yellowish green tinged with the purplish blue of anthocyanin pigments, and the uppermost

TABLE IX

MINUS-NITROGEN AND MINUS-SULPHUR TOMATO PLANTS SHIFTED FROM DAYLIGHT TO CONTINUOUS DARKNESS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND DATE			
	MINUS-N (PLUS-S)		MINUS-S (PLUS-NO_3)	
	JUNE 6	JUNE 18	JUNE 19	JULY 2
	NORMAL LIGHT	PART OF JUNE 6 PLANTS AFTER 12 DAYS' DARKNESS	NORMAL LIGHT	PART OF JUNE 19 PLANTS AFTER 12 DAYS' DARKNESS
Total sulphate-free S	0.024	0.015	0.024	0.019
Protein S	0.018	0.009	0.004	0.004
Sulphate-free soluble S	0.006	0.006	0.020	0.015
Sulphate S	0.011	0.018	Trace	Trace
Total S	0.035	0.033	0.024	0.019
Total nitrate-free N	0.129	0.109	0.218	0.211
Protein N	0.102	0.068	0.123	0.103
Nitrate-free soluble N	0.027	0.041	0.095	0.108
Basic N	0.008	0.008	0.014	0.021
Amino N	0.013	0.021	0.035	0.049
Amide N	0.005	0.014	0.022	0.016
Ammonia N	None	Trace	0.017	0.013
Other N	0.001	−0.002	0.007	0.009
Nitrate N	None	None	0.032	0.032
Total N	0.129	0.109	0.250	0.243

leaves and tip of the stem are rather dark green and may remain so for a considerable period. On the other hand, calcium-deficient tomato plants (**31**) are distinctive in appearance, in that the upper half of the plant is yellow rather than green, and the lower half instead of being yellowish is rather dark green. The lower leaves often remain green even after death of stem tips and complete loss of chlorophyll from the upper half of the plant.

TABLE X

PAPER-WHITE NARCISSUS BULBS GROWN IN SAND CULTURE FROM NOVEMBER 16 TO DECEMBER 13*

SULPHUR AND NITROGEN FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND PART OF PLANT					
	MINUS-N (PLUS-S)			COMPLETE (PLUS-NO_3)		
	CENTERS AND TOPS	STORAGE TISSUE	ROOTS	CENTERS AND TOPS	STORAGE TISSUE	ROOTS
Total sulphate-free S	0.150	0.200	0.280	0.255	0.220	0.220
Sulphate S	0.130	0.130	0.720	0.075	0.140	0.780
Total S	0.280	0.330	1.000	0.330	0.360	1.000
Total nitrate-free N	2.940	1.330	3.075	3.340	1.990	4.925
Nitrate N	None	None	None	None	Trace	0.845
Dry matter	11.300	30.080	6.030	10.000	27.270	6.470

* Results have been reported of analyses of these bulbs for carbohydrate and nitrogenous fractions (**28**).

TABLE XI

PAPER-WHITE NARCISSUS BULBS GROWN IN SAND CULTURE FROM NOVEMBER 16 TO DECEMBER 13

SULPHUR AND NITROGEN FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND PART OF PLANT					
	MINUS-N (PLUS-S)			COMPLETE (PLUS-NO_3)		
	CENTERS AND TOPS	STORAGE TISSUE	ROOTS	CENTERS AND TOPS	STORAGE TISSUE	ROOTS
Total sulphate-free S	0.017	0.060	0.017	0.026	0.060	0.014
Sulphate S	0.015	0.039	0.043	0.007	0.038	0.050
Total S	0.032	0.099	0.060	0.033	0.098	0.065
Total nitrate-free N	0.332	0.400	0.185	0.334	0.543	0.319
Nitrate N	None	None	None	None	Trace	0.055

Sulphur-deficient tomato plants, however, are easily distinguished from those lacking calcium. In the sulphur-deficient plants of these experiments the stem tips of the former, although small in diameter, were not dead nor even injured, and the upper portion of the plant was darker green than the lower. In some respects these plants were similar to plants deficient re-

spectively in nitrogen, phosphorus, and potassium, in that the stems and petioles were stiff and woody, anthocyanin was conspicuously present, and the foliage was light yellowish green, particularly on the lower half of the plant. They exhibited an apparently distinctive and remarkable capacity for stem elongation (fig. 1). Many of the plants lacking sulphur had even longer stems and were taller than those which received the complete nutrient solution. Yet the stems were much more woody, and, as shown in figure 1, were of extremely small diameter.

Low-nitrogen plants (**9, 20, 29**) never exhibit such a growth response unless they are subjected to some such treatment as darkness, shading (**26, 29**), or heavy pruning (**20**); and apparently phosphorus- (**10**) or potassium-deficient tomato plants exhibit rapid stem elongation only following or accompanying carbohydrate depletion, which often occurs only shortly before death of the plant.

PROTEOLYSIS.—The breaking down of proteins to simple water-soluble organic nitrogenous compounds as amino acids and asparagine, and the reutilization of these and similar compounds in the development of new tissues, is a process in plants that commonly follows or accompanies increase in moisture content and decrease in percentage of dry matter and stored carbohydrates (**8, 26, 29**). In seed germination there is available a conspicuous example of this process. There are present conditions of abundant moisture, and in the seed there occur continual decrease in stored carbohydrates or fat and rapid breaking down and utilization of storage proteins.

Nitrogen-deficient tomato plants are high in carbohydrates and obviously low in total nitrogen (**9, 20, 29**). Further, much of the nitrogen consists of complex relatively immobile storage proteins (**29**). Breaking down of these proteins apparently does not occur except when preceded or accompanied by loss of carbohydrates, as when the plants are placed in continual darkness (**29**). There then occur rapid stem elongation, decrease in complex storage proteins, and increase in percentage of the simpler water-soluble forms of organic nitrogen.

Tomato plants lacking an external potassium (**30**) or phosphate (**10**) supply are, except during the last stages of growth, high-carbohydrate plants; and there is little proteolysis or vegetative extension unless the plants are shaded, placed in darkness, or otherwise treated to decrease the carbohydrate reserves. However, shortly before death of potassium- or phosphate-deficient plants, there occurs a decided decrease in carbohydrates, accompanying which there is, apparently, considerable breaking down and utilization of protein and fairly rapid stem elongation. Death of the plant within a few days may be caused, due in part to deficiency of proteins of the right quality rather than to lack in total organic nitrogen

(**10, 30, 31**). Similar conditions may obtain in calcium-deficient plants, except that effects of lack of this element (**30**) are so sudden and drastic that even if carbohydrates are low, the period or extent of proteolytic activity is necessarily very limited.

Sulphur-deficient plants were at all times very high in carbohydrates (page 575); yet the stems of these plants (fig. 1) increased in length, although not in diameter, fully as rapidly as the plants which received the complete nutrient solution. Analyses (tables II and III), however, show that as compared with the complete-nutrient plants, the minus-sulphur group contained on July 8 more than twice the concentration of soluble organic nitrogen and were especially high in amino acids, amide, and ammonia. Further, the organic sulphur in the complete-nutrient plants was nearly all in a complex protein form; whereas, in the minus-sulphur series, it was mainly water-soluble, protein-free organic sulphur, only a very small part of which was cysteine or glutathione.

The initial tomato plants, which were strongly vegetative and actively growing, contained much soluble organic sulphur and gave a strong S-H reaction (page 570). This is in striking contrast to the quality of the soluble organic sulphur of the minus-sulphur plants, which included practically no S-H sulphur. The low concentration of soluble organic sulphur in the only moderately vegetative plus-sulphur plants is not entirely without precedent.[5] AITKEN (**1**) analyzed fresh blade tissue of five different grasses and states that the water-soluble "protein" fraction did not contain sulphur. It should be mentioned, though, that his method of harvesting for analysis resulted in an analytical sample of grass blades which apparently did not include active basal growing tissues. Likewise in apple (**22, 36**), in *Fagus* (**7**), and in *Salix* (**36**), limited data give some suggestion that sulphur in these plants may be in large part insoluble, especially in mature leaves and storage tissue.

As usual, the plants which received sulphate but no nitrogen in the nutrient solution (minus-nitrogen plus-sulphur series) were very high in carbohydrates, and were making no measurable growth when exposed to seasonal light conditions of the greenhouse; but during the period of darkness accompanying decrease of carbohydrates, the stem increased in length by 15 to 20 cm., protein decreased, and the concentration of soluble organic nitrogen approximately doubled (tables VIII and IX). The changes in sulphur fractions are less, but indicate definitely a marked de-

[5] Drying plant tissue may result in a marked increase in soluble organic sulphur and a corresponding decrease in protein sulphur. Various workers have used dried plant tissue in making analyses for these fractions, but the results would appear impossible of interpretation so far as protein and soluble organic sulphur are concerned.

crease in sulphur-containing proteins. (Stems only were analyzed; soluble organic sulphur formed proteolytically was presumably translocated to other organs.)

In contrast it would seem that the sulphur-deficient tomato plants were very actively breaking down sulphur-containing proteins, even under conditions of extreme carbohydrate accumulation. The effects of treatment in darkness on the minus-sulphur plants are in complete harmony with these results. When minus-sulphur plants were placed in darkness, there was a marked drop in carbohydrates but no perceptible change in rate of stem elongation as compared with minus-sulphur plants continually in the light, and no material change in concentration or quality of sulphur fractions (tables VIII and IX). Expressed on a green-weight basis, it is seen that changes in nitrogenous fractions were also slight, although, owing to loss in dry matter, the percentage of nitrogen as well as of sulphur shows an increase.

Proteolytic activity in plants (**8, 26, 29**) seems generally to be intimately associated with decrease in storage carbohydrates. Obviously, however, proteolysis in the sulphur-deficient tomato plants was not limited by the presence of a high concentration of carbohydrates. An explanation is not apparent, but it was this capacity on the part of the sulphur-deficient tomato plants which made possible extensive reutilization of organic sulphur.

Whether or not sulphates may be formed proteolytically is uncertain, although there is some evidence to indicate that this may occur (page 591).

COMPOSITION IN RELATION TO ANATOMY.—Tomato plants have previously been grown in a deficiency of nitrogen (**20, 29**), phosphorus (**10**), potassium (**30**), and calcium (**31**). At various stages of growth, and under different conditions of environment and nutrition, the concentration of carbohydrates has been found to vary widely. Accumulation of carbohydrates, whatever the cause, has been found to be associated with thick cell walls in xylem, phloem region, and cortex; whereas a low concentration of carbohydrates has been found to be associated with comparatively thin walls in these tissues. Similar conditions have been reported by WELTON (**47**) for oats and wheat. Tomato plants grown in a deficiency of sulphur are no exception to this generalization. The minus-sulphur plants of the present experiments were very high in carbohydrates; even the pith cells of the stem tip were thick-walled, although not so thick as the conductive elements of the xylem, fibers of the phloem region, and collenchyma of the cortex. It would seem that the thickness of cell walls is intimately associated with the supply of available carbohydrates, and only indirectly (with

the possible exception of calcium[6]) with the supply of various essential elements obtained from the soil.

On the other hand, the characteristics of the protoplast appear to be rather definitely modified by nutrient treatment. Plants low in nitrogen, but with opportunity for abundant photosynthetic activity, have little meristematic tissue but are not injured. A tomato plant may, under advantageous conditions, remain alive for at least two years without external application of nitrogen. The protoplasm, where present, gradually becomes less dense but is highly refractive and does not look opaque. Proteinaceous materials of the protoplasm may be extensively reutilized in the formation of new meristematic tissue; but this apparently occurs, as already mentioned, only when accompanied by a decrease in carbohydrate reserve.

This description applies equally well to minus-sulphur tomato plants, with the single exception that in sulphur deficiency, even though carbohydrates are high, sulphur-containing nitrogenous constituents of the protoplast are constantly being reutilized for the development of progressively smaller stem and root tips. In either nitrogen or sulphur deficiency, proteinaceous protoplasmic material is limited in amount, but there does not appear to be radical disintegration of the protoplast, as is found in phosphorus (**10**) and calcium (**31**) deficiency, nor deposition of the abnormal granular proteinaceous particles frequently observed in plants lacking potassium (**30**), phosphorus (**10**), or calcium (**31**).

It would seem from the results of these experiments with tomato, and from the work of others (**16, 17, 18, 46, 48**), that cysteine and glutathione or similar compounds are essential constituents of actively dividing cells. At least, meristematic tissues were not observed that did not contain higher concentrations of S-H sulphur than adjacent mature cells. It should be emphasized, however, that many other materials are equally essential. In fact, as already mentioned, sulphur deficiency in tomato was less drastic in its effect than limitation of other essential elements.

NITRATE ASSIMILATION AND CARBOHYDRATE ACCUMULATION.—Some tomato plants were grown with no external supply of either sulphate or nitrate. They were shifted to a solution containing nitrate but no sulphate, and the plants absorbed nitrate instantly. This experiment demonstrates that for nitrate absorption the presence of sulphate in a solution is unnecessary. The plants lacking sulphate and nitrate in their nutrient solution soon became

[6] Calcium deficiency in algae has been said (**35**) to prevent the formation of the middle lamella. In tomato (**31**), however, lack of calcium did not prevent the early formation of the middle lamella for which calcium apparently may not be directly necessary. Calcium pectate at later stages may, however, be essential. Especially in the fibrous roots of tomato, there was found a secondary effect of calcium deficiency that resulted in dissolution of the middle lamella and separation of cells.

exhausted of the nitrate initially present in the plant. Sulphate, however, was retained as such, and, following complete assimilation of nitrate, did not materially decrease in concentration. As might be anticipated, it was found practically impossible to obtain plants simultaneously deficient in sulphate and nitrate. However, absorption of nitrates does not appear to have been a limiting factor in the minus-sulphur tomato plants of these experiments, as they were consistently much higher in nitrate than the plants which received the complete nutrient solution (tables II and III).

Nitrates accumulated in the sulphur-deficient plants because the assimilation or synthesis of nitrates to amino acids and other organic nitrogenous compounds was greatly inhibited. ECKERSON (**13**) found that the tomato plants lacking sulphur were low in reducase (nitrate reducing material); the reducase was maintained at low level, allowing continuous but slow reduction of nitrate. Also in a few hours following the shift from minus- to plus-sulphur nutrient solution, the plants gave strong reactions for nitrites; and eleven days after receiving sulphate there was a marked decrease in carbohydrates. Carbohydrate accumulation is not unfavorable to nitrate reduction; in fact, typical low-nitrogen plants are very high in carbohydrates, and especially high in reducase activity (**9**). Accumulation of carbohydrates was not caused by the fact that sugars could not be translocated, nor accumulation of starch by the fact that digestion to sugars was seriously limited. When the plants were placed in darkness, starch was freely hydrolyzed to sugars. Starch and sugars were also present in very large quantities in all parts of the plant, even in stem and root tips. Obviously, translocation of carbohydrates could not have been more complete.

One of the principal uses of carbohydrates, however, is in protein synthesis. Carbohydrates and nitrates (tables II and III) undoubtedly accumulated in the sulphur-deficient plants because there was little synthesis of amino acids or other proteinaceous materials. In this respect, the low-sulphur plants are similar to tomato plants lacking phosphates (**10**) or potassium (**30**), although the response differs in degree (**13**).

NITRATE AND SULPHATE ASSIMILATION.—The assimilation of nitrates in plants (**9**) involves reduction to nitrates and ammonia, following which there is oxidation of sugars and synthesis of amino acids. In the tomato this process appears to be carried on in the more alkaline phloem region of roots and tops. In certain other plants, such as apple (**11**), narcissus (**28**), and asparagus (**26**), the reduction of nitrates is restricted mainly to the fibrous roots; and under favorable conditions of growth, nitrates are seldom found in the tops of these plants because they are synthesized to amino acids and other organic nitrogenous compounds in the roots.

An attempt was made to determine whether sulphate and nitrate assimilation were in any respect similar. ECKERSON found that the minus-sulphur

tomato plants were very high in material that reduced sulphates to sulphites. Likewise, when the minus-sulphur plants were shifted to plus-sulphur treatment, sulphites appeared in considerable quantity, especially in the phloem of the tops and to a less extent in similar tissue of the roots. Shortly following the appearance of sulphites, unusually strong reactions were obtained for S-H sulphur, including cysteine. Glutathione also was probably present. The S-H sulphur reactions were largely restricted, however, to the phloem and cambium and to the meristematic tips of stem and roots, a condition that has frequently been observed by others (**16, 17, 19, 48**). No cystine was observed. Undoubtedly there were present other unidentified compounds of soluble organic sulphur such as possibly methionine, a comparatively recently discovered sulphur-containing amino acid (**3, 25**). There was certainly much soluble organic sulphur in the minus-sulphur plants (tables II and III), even more than before shifting to plus-sulphur treatment; but practically none of it was in the form of cysteine, glutathione, or cystine.

In tomato, therefore, sulphate and nitrate reduction seem in certain respects to be similar, in that plants deficient in sulphur are most active in sulphate reduction, and plants deficient in nitrogen are especially active in nitrate reduction. The region of reduction in both cases is the phloem, and may take place in either roots or tops.

It has been pointed out that in asparagus nitrate reduction takes place in the roots and that nitrates are not usually found in the succulent actively growing stems or spears. Some spears were analyzed and found to contain, on a green-weight basis, 0.036 per cent. sulphate sulphur and traces of sulphite. The spears contained no nitrates. Some potted asparagus plants were placed in a chamber in darkness at 10° C. As usual (**27**) this temperature prevented assimilation of nitrates and they appeared in the spears, but there was no increase in concentration of sulphates. A field-grown apple tree was examined and found to contain nitrates in the fibrous roots only, yet sulphates and sulphites were found in roots, twigs, petioles, and leaves. Likewise, ECKERSON found that in sulphate reduction the tops of apple trees were even more active than the roots, although the reverse was true for nitrate reduction. A sample consisting of twigs and petioles was found to contain, on a green-weight basis, 0.018 per cent. sulphate sulphur, but no nitrates. It is apparent from tables X and XI that narcissus contains nitrate in the roots only, whereas sulphate is present in all parts of the plant.

In these plants, therefore, sulphate assimilation does not appear to be restricted to the roots. Most of the assimilation of nitrates, however, takes place in the roots.

GROWTH OF TOMATO AS ASSOCIATED WITH CONCENTRATION OF NITRATE AND SULPHATE.—Tables IV and V show that the leaves and fruits of the minus-sulphur plants were comparatively low in sulphate-free sulphur. This is not true of the stems and roots, however, especially on a percentage of green-weight basis. Likewise, the data of tables II and III show that the whole stems of minus-sulphur plants were, at all times during the experiment, practically as high in concentration of total organic sulphur as the plants which received and contained an abundance of sulphate.

The sulphur-deficient plants certainly were not low in total soluble organic sulphur (tables II and III) obtained apparently exclusively through the breaking down of proteins (page 583). However, in spite of the high concentration of total soluble organic sulphur, the plants contained practically no cysteine or glutathione. If these or other essential sulphur-containing materials are formed chiefly through sulphate reduction and not proteolytically, it would seem to explain the apparent need for maintaining in the tomato plant, during its period of active growth, a somewhat high concentration of sulphate.

Repeated experiments (**20, 29, 30**) involving nitrogen nutrition with nitrates as the sole source of nitrogen show that, for vigorous growth of tomato, there must be maintained in the plant a high concentration of nitrates. The concentration must be far in excess of that which will be assimilated or the plant does not grow vigorously. Nitrates in themselves, however, are not essential for growth; a vigorous nitrate-free tomato plant may easily be obtained by maintaining a lower percentage of ammonium (**42**). It may be that in nitrate or sulphate nutrition of tomato, a considerable quantity of the unelaborated ion must be present to furnish the plant at sufficient rate an adequate amount of certain intermediate products of protein synthesis. In this connection it may be pointed out that HAMMETT (**17**) has shown that low concentrations of S-H compounds in a nutrient solution greatly accelerated growth of roots of seedlings of *Zea mays* and *Phaseolus vulgaris*. It appears probable that in plant nutrition S-H sulphur bears a relationship to sulphate in some respects similar to that of ammonium to nitrate.

There appears to be no information available as to the possible physiological value of an abundance of sulphate[7] and nitrate in tomato. It is certain, however, that lack of sulphate and nitrate cannot be a limiting factor in the synthesis of organic compounds for which nitrogen and sulphur are necessary, so long as sulphate and nitrate are present in abun-

[7] Sulphates do not appear to be held by proteins or other ampholytes of tomato as tenaciously as some other ions, as for example calcium (**31**), nitrate (**42**), or ammonium ions (**42**). Electrodialysis (**23**) of tomato stem tissue failed to remove more sulphate than the usual method (**5**) of aqueous extraction at approximately pH 5.6.

dance. The initial plants, which were high in sulphate and nitrate (tables II and III), gave exceptionally strong S-H reactions, contained some cystine (page 570), and for complete-nutrient plants were comparatively high in sulphate-free soluble sulphur. There are, however, analyses (**18**, **32**, **41**) tending to show that the sulphate content of plants may be low, especially when the plants are nearly mature (**41**). Whether or not a strongly vegetative tomato plant might be obtained that was low in sulphate seems doubtful. It would have been worth while to have determined effects of giving tomato plants a nutrient solution containing no sulphate but instead a low concentration of S-H sulphur continually supplied. Chemicals in sufficient quantity for such nutrient treatment were not available, however. At least it is clear that the tomato plants of these experiments decreased greatly in vegetative activity before there was extreme depletion of sulphate (fig. 1, tables II and III).

On the other hand, high-protein narcissus bulbs (**28**) containing no nitrates, and with no external source of nitrogen, may grow vigorously for months with a nitrogen supply obtained solely through the utilization of storage proteins. The dormant narcissus bulbs which were studied contained, however, considerable quantities of sulphate (tables X and XI).

It was thought that seeds might be obtained which were free of sulphate and that with them a test might be made to see whether cysteine or other S-H compounds were formed proteolytically. Soy beans were employed, and, although the dormant seeds contained insufficient sulphate for a macro-determination (**2**), the benzidene reaction indicated traces of sulphate. There appeared to be practically no S-H sulphur in the dry seeds. Three hours after integuments had been broken and the seeds had been moistened with distilled water, there were noticeable quantities of sulphate and sulphite (benzidine and tetrathionate reactions respectively, page 568). Fairly strong reactions for S-H compounds were also obtained at that time, particularly in the region of the hypocotyl and epicotyl. The germinating seeds were watched at frequent intervals for a period of five days, but there was no noticeable change in concentration of sulphite or S-H sulphur. But at the end of the five-day germination trial, the quantity of sulphate sulphur in the seeds was found by macro-analysis (**2**) to be 0.006 per cent. This percentage figure is based upon the original air-dry weight of the seeds.

So far as the observations on reduced sulphur are concerned, these results are in agreement with those of VIVARIO and LECLOUX (**45**). The question as to whether S-H compounds were formed synthetically from sulphites or wholly or in part through proteolysis may not be answered at this time. Nitrates appear never (**9**, **26**, **27**, **28**, **29**) to be formed proteolytically, yet phosphates and probably ammonium may be formed from the breaking down of phosphatides (**10**). The presence of sulphatides (**21**)

has not been demonstrated in plants. However, catabolic changes in organic sulphur-containing compounds would appear to be a possible explanation of the appearance of sulphates in the germinating seeds of soy beans.

In this connection attention may be called to the fact that traces of sulphate (tables II and III) were present in the minus-sulphur tomato plants. Even as late as July 8, an occasional crystal of benzidene sulphate formed on addition of the benzidene reagent to sections of the stem tips of the sulphur-deficient plants. There was, however, no sign of disorganization of the protoplast. In low-phosphorus tomatoes, breaking down of phosphatides to phosphates is accompanied by definite injury to the cells, and death soon follows (**10**).

Summary

1. Symptoms of sulphur deficiency in tomato developed slowly in plants which lacked an external sulphate supply. The plants looked as though they had been gradually but not completely deprived of nitrogen. The lower leaves were yellowish green, the stems were hard and woody, and the roots were extensive. Both roots and stems were of very small diameter.

2. These characteristics may also be exhibited by tomato plants deficient respectively in nitrogen, phosphorus, or potassium. The sulphur-deficient tomato plants had, however, a remarkable capacity for stem elongation; and although the stems were woody and thin they increased in length, but not in diameter, as rapidly as the stems of the complete-nutrient plants.

3. The sulphur-deficient tomato plants were extremely high in carbohydrates, and contained much more nitrate than the plants which received the complete nutrient solution.

4. Carbohydrates and nitrate accumulated in the minus-sulphate treated tomato plants because reduction of nitrates and oxidation of sugars was comparatively slow although not entirely inhibited.

5. Digestion of starch and translocation of sugars took place freely in minus-sulphate tomato plants.

6. Cell wall thickness in tomato plants seems to be more intimately associated with carbohydrate content than with any other single factor of nutrition. The high-carbohydrate, minus-sulphate tomato plants had very thick cell walls and a relatively high proportion of fibers and lignified tissue.

7. Protoplasm of the sulphur-deficient tomato looked much like that of plants lacking nitrogen; that is, the protoplasm was limited in amount but not noticeably injured as in deficiency of phosphorus, calcium, or potassium.

8. Cysteine specifically, probably glutathione, and possibly other S-H compounds were present in meristematic tissue and were especially high

when complete-nutrient tomato plants contained an abundance of sulphate and nitrate.

9. Associated with low content of S-H sulphur in the minus-sulphate tomato plants there was practically no active cambium. Roots and stems were thus of small diameter, but they increased in length as a result of cell division in the apical meristems of stems and roots. The meristematic tissues contained faint traces of S-H compounds.

10. Proteolysis is usually accompanied by decrease in reserve carbohydrates. The minus-sulphate tomato plants were at all times extremely high in carbohydrates, yet complex proteins were rapidly broken down to soluble organic compounds of sulphur, to amino acids, to asparagine, and to ammonia. This proteolytic activity resulted, however, in little if any S-H sulphur.

11. Although the tomato plants lacking an external sulphate supply were not very low in percentage of total organic sulphur, much of it was water-soluble; whereas the organic sulphur of the complete-nutrient tomato plants was mainly in a complex protein form. The soluble organic sulphur in the minus-sulphur plants contained practically no cysteine, glutathione, or cystine. The form of sulphur was not determined.

12. Limited data give some evidence to indicate that sulphate and ammonium may be formed proteolytically. It is known that phosphatides break down in part to phosphates and probably ammonium.

13. In tomato, sulphate is reduced to sulphite and apparently to S-H sulphur in the comparatively alkaline phloem region of roots and tops. This is also true of the reduction of nitrates to nitrites and ammonium.

14. In apple, narcissus, and asparagus, the region of nitrate reduction is largely confined to the fibrous roots. Sulphate reduction in these plants takes place in the roots to some extent, but mainly in the tops.

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INFLUENCE OF POTASSIUM NITRATE ON NODULE FORMATION AND NITROGEN FIXATION BY CLOVER[1]

E. W. Hopkins, P. W. Wilson, and W. H. Peterson

Introduction

The unique position which the Leguminosae occupy in the plant world has made them particularly inviting subjects for investigation. The specialized structure arising from association with benevolent symbionts enables this group of plants to develop independently of the supply of nitrogen present in the soil. Not only do leguminous plants with associated bacteria require no added fixed nitrogen, but also the peculiar fact was long ago observed that nitrogenous compounds adversely affect this so-called symbiotic relation. Under these conditions there may be present large numbers of effective strains of the nodule bacteria, and yet the plant appears to resist their invasion or to make little use of its nodules. Thus it would seem that if both sources of nitrogen are available the plant prefers to feed upon fixed nitrogen in the soil. This subject has been investigated repeatedly, and several hypotheses have been proposed to explain this peculiar condition. However, the investigations have considered chiefly the effect of nitrate treatment on nodule formation, since that is the form in which plants usually take up their nitrogen, and because of its wide occurrence in soil. A second important form of soil nitrogen, *viz.*, ammonia, has been given far less attention; while organic nitrogen sources have been almost entirely neglected.

The major part of the work that has been done in this field has dealt with the effect of sources of nitrogen on the number of nodules. Inquiries that deal with the effect on the nitrogen fixation process are rare and often open to criticism. Thus the use of open pots for growing the plants is not satisfactory since growth of algae and other microörganisms would account for part of the nitrate that disappears. Obviously plants grown under bacteriologically controlled conditions are desirable if a satisfactory nitrogen balance is to be made. To grow plants free from contaminating organisms, it is necessary to adopt precautions that result in smaller plants than those grown in open pots. This leads to much smaller nitrogen uptake and the nitrogen balances are more likely to be affected by analytical errors. In spite of these objections it appeared desirable to investigate the formation of nodules and nitrogen fixation with plants grown under controlled conditions.

[1] Herman Frasch Foundation Research in Agricultural Chemistry, Paper no. 28. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

A second point that does not seem to have received sufficient consideration is the question whether the effects noted are due to the concentration of nitrogen at any one time, or to the total nitrogen added. It is possible that different results would be obtained if the nitrogen were added periodically rather than at one time.

RAUTENBERG and KÜHN (**11, 12**) appear to have been the first to observe that vetch (*Vicia faba*) formed no root nodules in solutions containing ammonia or nitrate nitrogen, while plants grown in nitrogen-free solutions possessed abundant nodules. DE VRIES (**13**) noted that red clover plants grown in nitrogen-rich solutions developed only a few nodules, as compared with plants without access to combined nitrogen. FRANK (**5**) found that horse-manure extract prevented formation of nodules by peas grown in a soil which had been ignited.

Historical review

It is not possible to give here a detailed review of the numerous papers which discuss the effect of nitrate treatment on nodule formation. A summary of these papers is given in the monograph by FRED, BALDWIN, and MCCOY (**6**). However, certain papers may be reviewed here, as they offer details not given in this monograph. FLAMAND (**4**) has made an extensive study of the concentrations of nitrate which inhibit nodule formation. Plants were grown in SACH's solution with various nitrates added. With peas (*Pisum sativum*), KNO_3, $NaNO_3$, and NH_4NO_3 were effective in dilutions of 1/10,000 in preventing nodule formation, while with $Ca(NO_3)_2$ a 1/2000 dilution was required. Vetch (*Vicia narbonensis*) exhibited no nodules in $NaNO_3$ 1/2000; KNO_3 1/10,000; $Ca(NO_3)_2$ less than 1/10,000; and NH_4NO_3 1/20,000. Horse bean (*Faba equina*) was somewhat less sensitive to some nitrates and more sensitive to others; KNO_3 1/10,000; $Ca(NO_3)_2$ 1/20,000; $NaNO_3$ 1/2000. $(NH_4)_2SO_4$ inhibited nodule formation on *Pisum sativum* and *Faba equina* at a dilution of 1/10,000 and on *Vica narbonensis* at 1/20,000.

WILSON (**14**) has studied the effect upon soybeans of many alkali and metal nitrates. An amount of these nitrates which had been found to have no injurious effect on the plant was added to 208 gm. of soil. The nitrates used were as follows: $Ca(NO_3)_2$, KNO_3, NH_4NO_3, $Mg(NO_3)_2$, $NaNO_3$, $Al(NO_3)_3$, $Ba(NO_3)_2$, $Fe(NO_3)_3$, $Pb(NO_3)_2$, $Ce(NO_3)_4$, $Sr(NO_3)_2$, $LiNO_3$, $UO_2(NO_3)_2$, 0.1 gm. added per 208 gm. of soil; $Cd(NO_3)_2$, $Ni(NO_3)_2$, 0.02 gm. per 208 gm. of soil; and $Zn(NO_3)_2$ and HNO_3, 0.05 gm. per 208 gm. of soil. $Al(NO_3)_3$ and HNO_3 had no effect upon the number of nodules, but all other nitrates exerted a marked depressing result. Of the ammonium salts tested, the carbonate was without effect, the phosphate and aluminum

ammonium sulphate were stimulating, while all others markedly decreased nodule production on soybeans.

ALBRECHT (**1**) found that the application of $NaNO_3$ corresponding to 10, 50 and 150 lb. of nitrate per acre did not appreciably decrease nodule formation by cowpeas, and that soybeans were not affected by amounts less than 150 lb. per acre.

GIÖBEL (**8**) has considered in some detail the effect of $NaNO_3$ upon soybeans and alfalfa. It was found that the gradual addition of the equivalent of 500 to 2600 lb. per acre of $NaNO_3$ to pot cultures of alfalfa was without result on nodule formation. When, however, the nitrate was all added at the beginning, 1200 to 1600 lb. per acre decreased the number of nodules to one fourth of normal. Amounts up to 1000 lb. of $NaNO_3$ were not effective. In another experiment soybeans were allowed to form nodules, and then $NaNO_3$ equivalent to 100 to 2100 lb. per acre was added gradually. The number of nodules was not consistently depressed, although the series containing about 300 lb. of $NaNO_3$ showed a decrease in weight of nodules. When the nitrate was added at the start, both the numbers and weights of nodules were decreased if more than 200 lb. of $NaNO_3$ per acre were applied. NH_4NO_3 was found to be twice as active as $NaNO_3$ in its effect upon the number of nodules. Generally, 100 to 200 lb. per acre of $NaNO_3$ greatly benefited the plant and stimulated nodule formation. GIÖBEL made nitrogen balances to determine whether nitrogen was fixed in the presence of the nitrate. He calculated the nitrogen fixed, by the difference between the nitrogen content of the tops of inoculated and uninoculated plants. Although the data show some fixation even when there was an excess of nitrogen added to the soil, GIÖBEL evidently did not consider this as significant, since he concludes: "Absorption of nitrates and fixation of atmospheric nitrogen by inoculated cultures took place simultaneously when the plants did not receive their full need of nitrogen from the soil. When this was the case, however, scarcely any fixation of nitrogen took place. In general the absorption of nitrates resulted in correspondingly smaller amounts of nitrogen being fixed. In other words the process of nitrate absorption dominated the process of nitrogen fixation." GIÖBEL'S conclusions are probably sound, but the data offered are not without serious objections. In the first place he used an indirect method for estimation of nitrogen fixed, and in the second place, as he says, not all of the nitrate nitrogen that disappeared in his cultures could be accounted for in the nitrogen in the tops. This difference was ascribed to nitrate used for root development and *microbial assimilation*. In one experiment he added the nitrate in one-fifth portions at intervals distributed over the growth period and compared the results with those obtained with cultures in which all of the nitrate was added at the start.

He states that no differences could be observed in nodule counts or dry weight of plants because of this difference in treatment.

OHKAWARA (**10**) grew lupine and serradella in small pots of sand. Different solutions containing 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1 per cent. of nitrate were added in the proportion of 80 cc. of solution to 400 gm. of sand. The 0.2 and 0.5 per cent. KNO_3 solution and 0.5 per cent. $NaNO_3$ solution prevented the formation of nodules on the plants. 0.2 and 0.5 per cent. $Ca(NO_3)_2$ solution inhibited nodule production on serradella, and the 0.5 per cent. on the lupines. The solutions exhibited no effect or were slightly stimulating from 0.0 to 0.02 per cent., but all higher concentrations exerted a depressing action on nodules. $(NH_4)_2SO_4$ solutions in the same concentrations as the nitrates were also tested. The 0.05 per cent. solution decreased nodule formation, and the 0.1 per cent. solutions were found to be effective in preventing nodules.

In summary, the quantities of alkaline earth nitrates and ammonium salts which are required to affect nodule formation may be briefly presented as follows. In water cultures, concentrations of 1/4000 to 1/200,000 of nitrate nitrogen have been reported to prevent nodules from forming. Ammonium salts have been found effective in concentrations of 1/2000 to 1/100,000. For field experiments, 100 to 300 lb. of nitrate per acre depress or inhibit the formation of nodules, although some workers report that 1000 lb. are necessary. Small amounts of nitrate, however, appear to favor plant growth, and sometimes to increase the number of nodules.

Experimentation

METHODS

The plant cultures were put up under aseptic conditions, and attempts were made to maintain sterility throughout the growth period. The plant used was mammoth red clover, which was set out in cotton-plugged 32-oz. round bottles containing the modified Crone's solution recommended by BRYAN (**2**). In all cases, 0.8 per cent. agar had been added to the plant culture solutions. This concentration of agar gave a gel of proper rigidity, but which was still soft enough to offer very little resistance to the plant roots. The amount of nutrient agar used in each bottle was 200 cc. in experiment I, and 250 cc. in experiment II.

The nitrate used throughout the work was KNO_3. In some cases, varying amounts of this compound were added to the agar before sterilization; in other cases a small amount of nitrate was introduced at the start, and additions were made at intervals. The cotton plug of each bottle was pierced with an 8-mm. cotton-plugged glass tube. The nitrate was added through this tube as a sterile solution with a sterile 1-cc. pipette.

The methods employed in sterilizing and planting the seeds have been described previously by HOPKINS, WILSON, and FRED (9).

For inoculation, a suspension of a pure culture of *Rh. trifolii* strain number 209 was used; this organism has been found to increase greatly the total nitrogen content of the host plant. During the period of growth in the greenhouse the plant cultures were covered with layers of absorbent cotton. These caps were sprayed with a 5 per cent. solution of sodium benzoate containing a bacteriostatic dye. This method of preventing contamination has since been replaced by the use of 10-oz. waxed drinking cups inverted over the tops of the bottles.

The total nitrogen of the plants was determined by the Davisson-Parsons procedure (FRED and WAKSMAN, 7 p. 68) which includes nitrate-nitrogen. The nitrate remaining in the agar was estimated by a modification of this method. The agar was melted and made up to 500 or 1000 cc. After cooling, 50 cc. of approximately normal potassium alum solution was added with agitation. By filtering, a solution practically free of agar was obtained. The nitrate in a suitable aliquot of this was determined by alkaline reduction with Devarda alloy and distillation into $\frac{N}{28}H_2SO_4$. The filtrates of several cultures in each series were tested qualitatively for the presence of NH_3 N and NO_2 N but none was found.

EXPERIMENT I

SERIES 1.—Potassium nitrate was added to the agar at the start in varying amounts, 1, 2, and 4 mg. of NO_3 N per 200 cc. The same quantity of nitrate as originally introduced into the agar was added at intervals of 2 weeks for a period of 10 weeks. Thus, one set of 5 bottles contained 1 mg. of NO_3 N and 5 additions of 1 mg. each were made; a second set contained 2 mg. of NO_3 N and 2 mg. were added five times; a third set contained 4 mg. of NO_3 N and 4 mg. were added five times. The inoculated controls received no nitrate.

When harvested at the end of 4 months the plants of the three sets were very similar in size and color to the controls; all of the cultures were somewhat pale. Distribution and number of nodules were determined on all of the plants; the results are given in table I. In the column headed *No. of nodules* is given the mean of each treatment with its standard deviation.

SERIES 2.—4 mg. of NO_3 N were present in the agar at the start (except in the control), and nitrate was added at the rate of 1 mg. per week for varying lengths of time. In the first set, only one addition was made; in the second, two; and so on up to the last set which received nine additions of 1 mg. each. The control is the same as that used in series 1. The plants, which were 4 months old when harvested, resembled the controls; all

TABLE I

EFFECT OF ADDITION OF POTASSIUM NITRATE PERIODICALLY ON NUMBER AND DISTRIBUTION OF NODULES

TOTAL NO_3 N ADDED	NO. OF SAMPLES	NO. OF NODULES PER 10 PLANTS	DIFFERENCE	PROBABILITY*	SIZE AND DISTRIBUTION OF NODULES
Control	8	124 ± 16	.....	.....	Principally on tap root, long type
Experiment I, series 1					
6 mg.	5	92 ± 5	32	0.15	Principally on tap root, both long and round
12 mg.	5	72 ± 8	52	0.04	Principally on secondary roots, mostly round and scattered
24 mg.	5	62 ± 12	62	0.02	Same as 12 mg. but smaller
Experiment I, series 2					
5 mg.	3	106 ± 7	18	0.50	Principally on secondary roots, mostly long type
6 mg.	4	96 ± 20	28	0.25	Same as 5 mg.
7 mg.	3	66 ± 20	58	0.07	Principally on secondary roots, mostly round and scattered
8 mg.	3	61 ± 9	63	0.05	Same as 7 mg.
9 mg.	2	77 ± 18	47	0.20	Same as 7 mg.
10 mg.	3	83 ± 11	41	0.20	Same as 7 mg.
11 mg.	3	70 ± 15	54	0.09	Same as 7 mg.
12 mg.	2	91 ± 9	33	0.35	Same as 7 mg.
13 mg.	3	93 ± 27	31	0.30	Same as 7 mg.

Series I. 1 mg. at start; 1, 2, and 4 mg. respectively added every 2 weeks for 10 weeks.

Series II. 4 mg. at start; 1 mg. added per week until designated quantity reached.

* Probability that observed difference could have arisen from random sampling.

attained a fair size but were pale green. The results for series 2 also are given in table I.

EXPERIMENT II

SERIES 1.—The nitrate was added in varying amounts, about 3, 5, 10, 15, 20, and 40 mg. per bottle (250 cc.) at the start of the experiment, and

no further addition was made during the growth period. The plants were harvested after seven weeks and examined for size and distribution of nodules as in experiment I. In addition, nitrogen determinations were made so that a nitrogen balance could be determined. In appearance, the high nitrate cultures were somewhat paler than either the low nitrate or the controls. The remainder of the data for this series are given in tables II and IV.

TABLE II

EFFECT OF ADDITION OF POTASSIUM NITRATE AT THE START ON NUMBER AND DISTRIBUTION OF NODULES

TOTAL NO_3 N ADDED	NO. OF SAMPLES	NO. OF NODULES	DIFFERENCE	PROBABILITY	SIZE OF DISTRIBUTION OF NODULES
Control	11	73 ± 4	.....	.....	Principally on tap root, mostly long type
Experiment II, series 1					
3.3 mg.	4	70 ± 11	3	0.75	Principally on secondary roots, round and scattered
5.4 mg.	3	52 ± 10	21	0.04	Both tap and secondary Round and scattered
10.1 mg.	3	59 ± 10	14	0.15	Principally on tap roots Round and scattered
14.8 mg.	3	49 ± 10	24	0.02	Same as 10.1 mg. but slightly smaller
19.8 mg.	3	60 ± 9	13	0.20	Entirely on secondary roots, all round, small and scattered
39.0 mg.	3	36 ± 5	37	<0.01	Same as 19.8 mg.

SERIES 2.—Nitrate was added in the same amounts as in series 1 (3, 5, 10, 15, 10, and 40 mg.) except that its addition was extended over a period of 5 weeks. Three mg. of NO_3 N were added at the start to all cultures except the controls. The remaining nitrogen was added at weekly intervals; *e.g.*, to the 5 mg. set, 0.4 mg. per week; to the 10 mg. set, 1.4 mg. per week, and so on. The same controls were used for series 1, 2, and 3. The plants receiving nitrate were a paler green than the controls, the color and size of the plant tending to be adversely affected by the increase in nitrate. However, the differences were not so marked that they require consideration in interpreting the results. The plants were harvested after 7 weeks.

The data on the nodules are given in table III and those on nitrogen fixation in table IV.

TABLE III

EFFECT OF ADDITION OF POTASSIUM NITRATE PERIODICALLY ON NUMBER AND DISTRIBUTION OF NODULES

TOTAL NO_3 N ADDED	NO. OF SAMPLES	NO. OF NODULES	DIFFERENCE	PROBABILITY	SIZE OF DISTRIBUTION OF NODULES
Control	11	73 ± 4	.....	.....	Principally on tap root, mostly long type
Experiment II, series 2[1]					
3.3 mg.	5	54 ± 4	19	.01	Principally on secondary roots; both round and long, scattered
5.4 mg.	5	48 ± 8	25	<.01	Principally on secondary roots; round and scattered
10.1 mg.	5	43 ± 7	30	<.01	Same as 5.4 mg.
14.8 mg.	5	45 ± 8	28	<.01	Same as 5.4 mg. but smaller
19.8 mg.	5	22 ± 4	51	<.01	Same as 5.4 mg. but a few long type
39.0 mg.	5	27 ± 5	46	<.01	Same as 5.4 mg.
Experiment II, series 3[2]					
2.6 mg.	5	78 ± 12	5	0.60	Principally on secondary roots, both long and round, scattered
4.2 mg.	5	69 ± 12	4	0.70	Same as 2.6 mg.
7.4 mg.	5	65 ± 18	13	0.50	Principally on secondary roots, mostly round and scattered
12.2 mg.	5	54 ± 13	24	0.08	Same as 7.4 mg.
15.4 mg.	5	33 ± 3	45	<.01	Same as 7.4 mg.

[1] Series 2. 3.3 mg. NO_3 N at start. Remainder added in aliquots, weekly, for five weeks.

[2] Series 3. 2.6 mg. at start. Remainder added weekly at rate of 1.6 mg. per week.

SERIES 3.—It was intended that the additions of nitrate in this series should be in 2-mg. quantities as in series 1 and 2. However, on analysis of control cultures, *i.e.*, unplanted but receiving the same addition of NO_3 N it was found that the solution added each week contained 1.6 mg. NO_3 N instead of 2.0 mg. For this reason the total quantities of NO_3 N added in this series are somewhat less than the corresponding ones in series 1 and 2 of this experiment. In this series 2.6 mg. of NO_3 N were added to all the cultures at the start, then 1.6 mg. were added weekly until the following quantities were reached: 4.2, 7.4, 12.2, 15.4 mg. of NO_3 N. Thus only one addition was made to the 4.2 mg. set and eight additions were made to the 15.4 mg. set. At the time of harvesting (8 weeks) the plants

TABLE IV

EFFECT OF ADDITION OF POTASSIUM NITRATE UPON NITROGEN FIXATION

NO_3 N ADDED	No. OF SAMPLES	NO_3 N IN AGAR AT END	NO_3 N USED	NITROGEN* IN SIX PLANTS	NITROGEN FIXED	PROBABILITY†
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	
Control	11	0.0	0.0	4.1	4.06 ± 0.21	
Experiment II, series 1						
3.3	4	0.0	3.3	5.4	2.08 ± 0.55	0.04
5.4	3	0.2	5.2	6.9	1.70 ± 0.35	0.04
10.1	3	3.5	6.6	7.6	1.00 ± 0.34	0.10
14.8	3	8.3	6.5	7.5	1.00 ± 0.34	0.10
19.8	3	12.2	7.6	8.2	0.6 ± 0.53	0.35
39.0	3	31.9	7.1	6.5	−0.6 ± 0.60	0.45
Experiment II, series 2						
3.3	5	0.0	3.3	5.6	2.28 ± 0.16	<0.01
5.4	5	0.1	5.3	6.1	0.78 ± 0.25	0.04
10.1	5	3.5	6.6	7.5	0.94 ± 0.52	0.15
14.8	5	9.1	5.7	6.8	1.14 ± 0.51	0.10
19.8	5	13.8	6.0	6.2	0.20 ± 0.90	0.80
Experiment II, series 3						
2.6	5	0.0	2.6	5.8	3.20 ± 0.26	<0.01
4.2	5	0.0	4.2	6.5	2.28 ± 0.28	<0.01
7.4	5	0.4	7.0	8.0	1.06 ± 0.35	0.04
12.2	5	4.2	8.0	7.7	0.30 ± 0.20	0.20
15.4	4	9.2	6.2	7.1	0.90 ± 0.25	0.05

* Corrected for uninoculated controls.

† Probability that observed fixation could have resulted from random sampling.

receiving the higher quantities of nitrogen (12.2 and 15.4 mg.) were paler in color than the remaining sets. The data are given in tables III and IV.

Discussion

The data given in the tables are best suited for discussion as a whole rather than individually; for this reason interpretation of the results of the various experiments has been reserved for this section of the paper.

Effect of added nitrate on number of nodules

In experiment I (table I) the number of nodules on the control plants, *i.e.*, those receiving no nitrate, was extremely variable; it will be noticed that the standard deviation of the mean is about 13 per cent. of the latter. Unpublished experiments in which we have made a study of the distribution curve of nodule formation in agar under the same conditions as were used in these experiments have indicated that the standard deviation of the mean should be about 5 per cent. of the mean. Because of the high variance in this experiment, significant decrease in the number of nodules in those plants receiving nitrate is rather difficult to demonstrate. The observed differences were tested by the "Student" method as modified by Fisher (3). Our experience has indicated that nodule formation satisfies the fundamental assumption of this method, *viz.*, that the estimated standard deviation is independent of the mean. The 4th column of the table gives the probability that the observed difference could have arisen from sampling. This probability is taken from a table which is entered by calculation of the statistic t:

$$t = \frac{X_1 - X_2}{s}\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

$$s = \sqrt{\frac{\Sigma (X_1 - \overline{X}_1)^2 + \Sigma (X_2 - \overline{X}_2)^2}{n_1 + n_2 - 2}}$$

Where $\overline{X}_1$ is mean of n_1 observations (X_1), $\overline{X}_2$ is mean of n_2 observations (X_2); the table is entered at $n = n_1 + n_2 - 2$. A consideration of the formula shows that if the variance $\left[\frac{\Sigma (X_1 - \overline{X}_1)^2}{n_1}\right]$ of the controls is extremely high this will lead to large values of s independent of the variance in the treated samples. This large value of s will decrease the value of t which leads to a high probability that the difference arose from random sampling. For this reason it seems that any probability in this series of the order of 0.05 very likely indicates a significant decrease in the number of nodules. In series 1 the set in which 12 mg. were added at the rate of 2 mg. per week, and the set in which 24 mg. were added at the rate of 4 mg. per week,

clearly decreased the number of nodules. In series 2, many of the treated samples have a high variance combined with a small number of samples; this leads to erratic results. Those samples that received 8 mg. of NO_3 N showed significant decreases in the number of nodules but the others did not.

In experiment II, the variance of the controls was much less and very similar to that found in previous experiments. On the other hand, the treated samples very often have extremely large values for the variance. This might be expected, since the effect of nitrate appears to be due to the disturbance of a delicate equilibrium of a nutritional character between plant and bacteria, and wide variations in results of the same treatment are not surprising. Thus in series 1 in which the nitrate is added at the start, it appears that the 5.4 mg., 14.8 mg., and 39.0 mg. sets show a decrease in the number of nodules whereas the 10.1 mg. and 19.8 mg. sets do not. On examination of the original data for these two sets, it was observed that two of the three samples taken showed a significant decrease in the number of nodules formed but the third sample was not affected. This led to a large variance and consequently an apparent high probability that the observed difference arose from sampling. It is likely with larger numbers of samples that this erratic response would be eliminated to a certain extent. In series 2 (table III) in which the nitrate was added over a period of 5 weeks, all the sets exhibited less variability in response to the treatment. In every case, even the 3.3 mg. treatment, there was a significant decrease in the number of nodules formed in the presence of the nitrate. In series 3, wide variation among the replicates in a given treatment was again noticed. For example, the number of nodules per six plants in this series were: 2.6 mg., 36, 101, 70, 101, 80; 4.2 mg., 116, 67, 49, 56, 58; 7.4 mg., 131, 50, 48, 45, 52; 12.2 mg., 101, 40, 59, 34, 38; 15.4 mg., 40, 35, 39, 28, 23. It will be noticed that in each set except the 15.4 mg., four of the five replicates checked very well in the response to nitrate addition but the 5th culture gave a large increase in the number of nodules which led to an increase in both mean and variance for the set. Consequently the effect on four of the cultures was obscured by the unusual behavior of a single culture. Calculation of the probabilities for this series, with the elimination of the erratic culture, gave: 2.6 mg., 0.09; 4.2 mg., 0.05; 7.4 mg., 12.2 mg., and 15.4 mg., < 0.01. Therefore it appears that in four of the cultures in all sets receiving more than 2.6 mg. of NO_3 N, addition of the nitrate decreased the number of nodules; the remaining culture showed an unexplained stimulation in nodule formation.

In general it seems that about 5 mg. of NO_3 N per 250 cc. is sufficient to cause a decrease in the nodule formation of clover plants grown in agar. There is also an indication that, as the quantity of NO_3 N is increased,

there is a decrease in number of nodules formed, but this decrease is too variable to be of a linear nature. As far as could be determined, it made little difference in the response of the plant whether the addition of the nitrate was made at the start or periodically. Series 2, experiment II, indicated that addition of the nitrate over a 5 weeks' period was more effective than the other treatments, but this is without doubt partially due to the smaller variability in the data for this series as compared with the others. Finally it is to be noted that in none of our treatments was nodule formation entirely suppressed. The highest concentration used (39 mg. per 250 cc.) corresponds to a concentration of about 1.6 parts per 10,000. This exceeds the concentration that has been reported by various authors as sufficient to prevent formation of nodules. It is very likely that the quantity of nitrate nitrogen necessary for complete suppression of nodules on leguminous plants is variable, dependent on the growth of the plant, which in turn is dependent largely on experimental conditions used.

Effect of added nitrate on the size and distribution of the nodules

The use of the number of nodules as a criterion for evidence of suppression of the activity of rhizobia in leguminous plants is not entirely satisfactory. It was shown in these experiments that the variability in the response of the plants as measured by the number of nodules often obscures the interpretation of a treatment. Because of erratic individuals in a set, differences that are significant are not always apparent when a statistical test is used. Other workers encountering this have discarded the nodule count for other methods. Thus Gibbel used both fresh and dry weight of nodules as a measure of the response of soybeans to the addition of nitrates. He found that in many cases no significant decrease in the numbers of nodules could be discerned but that the weight of nodules was inversely proportional to the quantity of nitrate nitrogen added. Furthermore, there appeared to be a sharp correlation between mass of nodules and nitrogen fixed. While in our experiments it was not possible to weigh the nodules, observations on the size and distribution were made. As shown in tables I, II, and III, the effects of nitrate on size and distribution are extremely consistent. The nodules on the controls were located principally on the tap root, often near the crown; the majority of them were of the long finger-like type. The addition of small amounts of NO_3 N altered this. More of the nodules were found on the secondary roots and the round type of nodule was in evidence. As the quantity of NO_3 N added was increased, the nodules were found almost exclusively on the secondary roots and became progressively smaller and more scattered. This effect on size and distribution of nodules appeared to be fairly independent of the method used for adding the nitrate. However, it does appear that about

3 mg. per 250 cc. at the start is necessary to cause the distribution to change from the tap root to the secondary roots, since in series 1, experiment I, many of the nodules in the 6-mg. set were of the long type and on the tap root. In this set there was only 1 mg. of NO_3 N at the start and 1 mg. was added weekly. In the other series at least 3 mg. were present at the start and in all cases both the 3- and 5-mg. set showed the nodules distributed principally on the secondary roots. From these data it is apparent that observation of the size and distribution of the nodule is a decidedly more consistent method of measuring the effect of nitrate on inoculated plants than is the counting method, even though the results can be expressed only in a qualitative manner.

Effect of added nitrate on nitrogen fixation by the plants

The nitrogen balance for experiment II is given in table IV. These data show that even the smallest quantity of NO_3 N added was effective in decreasing the amount of nitrogen fixed by the association of organism and plant. However, in the presence of nitrate the total quantity of nitrogen found in the plant was increased so that a slight fixation did occur. For example, fixation took place in the 5-mg. set in every series, although it appeared from the controls that about 4.0 mg. were sufficient to meet the needs of the plants grown under these conditions. This suggests that the presence of the nitrate stimulates the plant to a certain extent and a better growth results. It is probable that this stimulation is due to the beneficial effect on the plant of a source of nitrogen during the period of nitrogen starvation, *i.e.*, before nitrogen fixation begins. Finally, it should be noted that in every case in which there is present an excess of nitrate there was no significant fixation. In the border line cases, *viz.*, about 10 mg., there often appears to be a slight gain (order of 1 mg.), but when the errors attendant on the nitrate determination in the agar are taken into consideration, this fixation cannot be considered significant. The results for the 15.4-mg. set in series 3 appear to indicate a slight fixation in this set. This might have been because the small quantity added each week (1.6 mg.) was insufficient for the needs of the plant in the early stages. However, since no fixation was observed in the 12.2-mg. set in this series, it is more likely that apparent fixation was obtained because of errors in analysis of the nitrate left in the agar.

From these data it is concluded that the addition of NO_3 N to inoculated plants does not prevent nodule formation by the bacteria but it does delay establishment of the bacteria in the host plant and modify their behavior toward the latter. Even the addition of quantities of NO_3 N that are much in excess of the requirements of the plants does not entirely prevent the formation of nodules; however, these nodules do not develop normally and

appear to act parasitically on the plant. A very delicate equilibrium must exist between the needs of the plants for nitrogen and the fixation process; in all cultures which were not supplied with sufficient nitrogen to meet the needs of the plant, fixation occurred, but as soon as an excess of nitrogen was added, the fixation process appeared to be entirely suppressed in spite of the fact that nodules were present. These effects were independent of whether the nitrate was added at the start or periodically to the plant culture. In the latter case, the addition of NO_3 N at the rate of 2 mg. per week was evidently sufficient to keep a supply available to the plant, if this addition was continued throughout the growth period.

Summary

1. The addition of nitrate nitrogen to clover plants grown in agar generally resulted in a decrease in the number of nodules formed when the concentration exceeded 2 to 3 parts per 100,000. However, the response of the plant to nitrate addition was erratic, and significant decreases were not always noted. Whenever decreases were observed, the effect appeared to be independent of whether the nitrate was added at the start or periodically. However, the most consistent depression of nodule formation was noted in the series in which the nitrate was added over a period of 5 weeks. Complete prevention of nodule formation was not effected by even the highest concentration of NO_3 N used, *viz.*, 1.6 parts per 10,000.

2. The distribution and size of the nodules were markedly affected by all the concentrations used. Addition of even small quantities of nitrate brought about formation of scattered nodules, largely of the round type, on the secondary roots; whereas normally the nodules are mostly of the long type and are found on the tap root near the crown. The size of the nodules was decreased as the quantity of nitrate was increased. These results were independent of the method of adding the nitrate provided a certain minimum quantity of nitrogen was available at the start.

3. All concentrations of nitrate nitrogen used markedly decreased the fixation of free nitrogen. If the quantities of NO_3 N added were insufficient for the needs of the plants, the deficit was made up by fixation of atmospheric nitrogen. However, as soon as an excess of nitrogen was provided, the fixation process appeared to be completely suppressed, even though nodules were present.

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INFLUENCE OF SOME ENVIRONMENTAL CONDITIONS ON THE GROWTH OF EXCISED ROOT TIPS OF WHEAT SEEDLINGS IN LIQUID MEDIA

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(WITH FOUR FIGURES)

Introduction

The experiments reported in this paper are of a preliminary nature, dealing with what may reasonably be expected from solution cultures of excised roots of wheat seedlings. In a certain sense (**11**) the culture of excised root tips may be considered as lying within the field of tissue culture, a field that has received much attention at the hands of animal physiologists and one that clearly deserves much more attention among plant physiologists than it has received thus far.

Materials and methods

Use of liquid media. The most satisfactory experiments on the culture of excised root tips thus far reported appear to be those of ROBBINS (**4, 5**) and ROBBINS and MANEVAL (**6, 7**), and the technique of the present study was largely based on the methods employed by these writers. Liquid media were used. Some authors, for example DIETRICH (**1**), KOTTE (**2**), and ÚLEHLA (**9**), have regarded liquid media as unsuited to such experiments as those here considered; but it is obvious that the use of liquid media avoids many complexities that are inevitable when water-impregnated solid media are employed. It is surely desirable, in approaching any new line of experimentation, to begin with the simpler kinds of systems, and the solution-culture method is relatively simple, with regard to the preparation of more or less known media as well as to the analytical interpretation of results. It is of course clear, although not always brought into biological discussion, that the indications of any experiment are directly applicable only to the set of conditions and circumstances under which the experiment was performed, and undue generalizations are to be avoided. The study here reported dealt only with the growth of these excised root tips in aqueous solutions. How similar root tips might behave in other media is of course a subject for direct experimentation.

Wheat seedlings were chosen as the source of experimental material since the physiology of the wheat plant is of special interest because of its great agronomic importance in many parts of the world, because wheat has already been studied in many ways, and because wheat seedlings

present relatively few difficulties of manipulation in such experimentation as is dealt with here.

The distilled water used in the nutrient solutions was finally prepared by triple distillation of spring water, first over alkaline potassium permanganate, then over barium hydroxide, and finally without any addition. The whole process was carried out in "Pyrex chemically resistant" glass and the water was stored in containers of the same material. This gave a very uniform product, of very low electric conductivity (about 3.4×10^{-6} reciprocal ohms), and without any apparent toxicity. Even with very nearly this same low conductivity, toxicity was evident in many instances when water distilled in other ways was tried.

The chemicals used were of high grade and no attempt was made to purify them or to take into account such traces of impurity as were present according to the makers' labels. The culture vessels were 125–ml. Erlenmeyer flasks, of "Pyrex chemically resistant" glass. Each was supplied with 50 ml. of medium, with a single root tip. Flasks were used repeatedly, always being cleaned by thorough rinsing, immersing in cleaning fluid (sulphuric acid and potassium bichromate), rinsing first in running hot water and then three times in ordinary distilled water and finally once in triple-distilled water, after which they were allowed to drain before charging.

The root tips used were taken from seedlings produced under standard conditions. A lot of seed supplied by a commercial firm showed a high proportion of defective seeds and much tardy germination, and most of this study was carried out with a supply of a "pure-line" Marquis wheat (C. I. no. 3641), kindly supplied by the United States Bureau of Plant Industry. The seeds used were selected by hand and eye with respect to size and general appearance, all that appeared unusual being discarded. These seeds were soaked in WILSON'S (**12**) calcium-hypochlorite sterilizing solution for 3–5 hours, in a bottle continually rotated at a speed of about 60 revolutions per minute. The treated seeds were thoroughly rinsed in heat-sterilized distilled water, after which they were distributed in heat-sterilized petri dishes (10 cm. in diameter), 12 seeds in each dish. On the bottom of each dish, beneath the seeds, were two sheets of filter paper (Whatman, no. 1) moistened with about 3 ml. of heat-sterilized distilled water. The seeds were evenly spaced on the paper circles in order to permit roots to develop without too many contacts. After the covers had been placed on them the dishes were set aside in a dark room with the temperature artificially maintained at about 25° C., and germination was allowed to proceed for about four days, until the first roots had attained a length of about 2 cm.

From the supply of apparently normal roots thus obtained, terminal 3-mm. tips were removed by means of a sharp scalpel, each tip being promptly introduced into its culture flask, which had previously been charged with the requisite solution. The excised portion included all of the meristematic region (which is translucent in these roots) and also about 1 mm. of the region of elongation (which is more opaque and more nearly white in appearance). These manipulations were performed with precautions designed to avoid the entrance of bacteria and fungus spores into the flasks. Serious troubles due to microörganisms were encountered in some instances, but obviously contaminated cultures were discarded. It was noted that tips might show injury, as if by traumatic shock, if the scalpel used had not been very sharp or if they had been handled carelessly. Injured tips failed to elongate promptly, although in many instances they appeared to have recovered after a day or two and then began to lengthen.

Excepting some cultures in very unfavorable media (such as distilled water), well prepared tips showed no initial retardation of elongation, and their length was about doubled in the first 24 hours after excision. In most of the solutions tested they maintained this growth rate for three or four days, after which the rate of elongation was apparently controlled by the nature of the surrounding solution. The initial period during which the characteristics of the medium generally failed to produce any notable effect on the rate of growth may perhaps represent the time required for the supply of some materials originally present in the tissues to become exhausted, but the delicate and difficult problem thus suggested was not attacked.

The basic mineral-nutrient solution contained the following six inorganic salts, with the concentrations given (in milligrams per liter): $Ca(NO_3)_2 \cdot 4H_2O$, 144.0 mg.; $MgSO_4 \cdot 7H_2O$, 50.0 mg.; K_2CO_3, 34.8 mg.; KNO_3, 25.0 mg.; KH_2PO_4, 25.0 mg.; $Fe_2(SO_4)_3$, 1.25 mg. This formula was used by USPENSKI and USPENSKAJA (**10**) in their studies on *Volvox*. It contains all of the ions that are generally essential, in considerable concentrations, for plant growth. By means of preliminary tests it was chosen from among seven more or less different solutions used for plant cultures by various experimenters, mainly because it was found to maintain itself without opalescence or precipitation throughout a wide range of H-ion concentration (from pH 5.0 to pH 9.0), while the others all failed in this respect. That the basic solution should show no precipitation throughout such a range was essential in the present study because it was planned to devote some attention to the influence of H-ion concentration on root-tip growth and many pH values would need to be tested. The USPENSKI solution is unusually dilute; its total salt concentration is less than one-seventh that of the KNOP-PALLADIN solution (**3**); and it is only about one thirty-sixth as

concentrated as the SHIVE (**8**) 3-salt solution (R_5C_2, osmotic value of 1.75 atm.). As the experimental results showed, the salts, salt proportions, and total concentration of the USPENSKI solution proved to be very suitable for the cultures here considered.

Dextrose and yeast extract were regularly added to the basic solution just described, since ROBBINS (**5**) had found them favorable to the growth of excised root tips. Dextrose was added in the proportion of 20 gm. per liter of solution and yeast extract in such amounts as to give a final concentration of an extract of 100 mg. of yeast per liter. The extract was prepared by boiling 0.5 gm. of dried brewers' yeast (obtained from Wallerstein, New York City) for half an hour in 100 ml. of distilled water. This was then filtered and allowed to cool, after which the filtrate was made up to the original volume. Twenty ml. of this extract added to a liter of the solution gave the required concentration. A fresh supply of yeast extract was prepared for each series of cultures. In all series the solutions were distributed immediately to their respective flasks, which were autoclaved for 20 minutes at a pressure of 1.25 atm. (20 lb. per sq. in.) and then set aside for 1–4 days before inoculation.

The general experimental conditions and procedures for all cultures of a series were nearly uniform. Four hundred simultaneous cultures constituted each series. The 400 flasks stood in 40 rows of 10 each, on a long table along the wall of a well ventilated basement room with maintained temperature of about 25°. Continuous electric illumination was regularly supplied by means of Mazda nitrogen-filled "daylight" lamps. Except in the experiments on H-ion concentration as such, no attempt was made to control the acidity of the media, but in almost all series the pH value of each modification of the culture solution was recorded, using a Youden quinhydrone-electrode potentiometer. Within each series a number of different conditional complexes were tested and each test was regularly repeated at least once. Results were accepted as valid only when two or more tests were in good agreement. The experimental period was two weeks in length. Throughout this period the culture flasks were kept closed with cotton plugs. The solutions were not renewed. Measurements of root length (in millimeters) were made only at the end of the experimental period. At that time the root tips were removed from the culture flasks and spread out under water to facilitate examination. For each culture the length of the main root, the length of all branch roots taken together, the number of branch roots, and the total length of main root and branch roots combined were recorded, together with notes on general appearance. For each set of conditions the mean of the total-length values of all roots grown under that conditional complex is taken as a numerical index of growth.

Experimental results

Water, mineral salts, dextrose, and yeast extract made up the full nutrient medium for these cultures, as has been said. With suitable temperature and illumination (see later) this gave a growth index of 121.1 mm. A representative root tip, grown 2 weeks in this medium at 25° and with artificial illumination, is shown at *A* in figure 1. Elongation and branching continued throughout the period and the root tips appeared healthy and vigorous at the end.

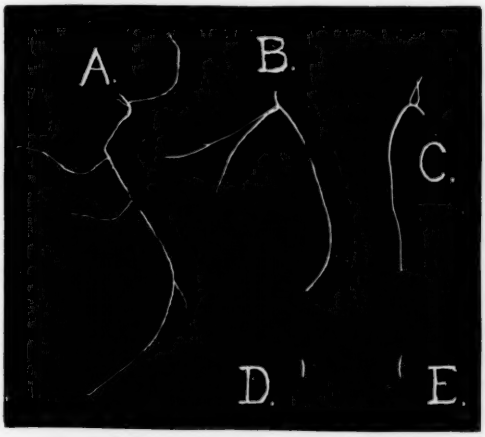

FIG. 1. Representative root tips grown two weeks at 25° and with continuous electric light, approximately natural size: *A*, cultures in full nutrient medium (redistilled water to which salts, dextrose, and yeast extract had been added); *B*, cultures in similar medium but without yeast extract; *C*, cultures in medium same as for *A* but without the salts. The two small roots shown at the bottom, without significant growth, represent cultures with a simple dextrose solution (*D*) and with redistilled water to which only yeast extract had been added (*E*).

An otherwise similar medium from which yeast extract had been omitted gave a corresponding growth index of only 72.0 mm. A representative root tip from this modified solution appears at *B* in figure 1. While growth here was somewhat marked and generally continued throughout the period, it was much less rapid than when salts, dextrose, and yeast extract were all supplied. Omission of yeast extract apparently reduced the growth index by about 40 per cent. in the 2-week experimental period; nevertheless the root tips appeared healthy at the end of the period.

Omission of the mineral salts from the full nutrient medium gave a still smaller growth index, only 42.3 mm., and permitted little or no branching.

Of course the solution thus modified supplied both dextrose and yeast extract, and its osmotic value was closely similar to that of the regular medium. A representative root tip from this solution is shown at *C* in figure 1. Much of the growth occurred in the early part of the experimental period and elongation soon became very slow or ceased altogether. Variability in extent and manner of growth was greater with this medium than with either of the two media just referred to, and the appearance of the root tips at the end of the experiment suggested poor health.

Distilled water with dextrose alone or with yeast extract alone gave only insignificant growth or none at all, and such slight elongation as did occur in these media was confined to the very early part of the experimental period. Nevertheless the root tips appeared to be still alive at the end of the period. The two small root tips (*D* and *E*) at the bottom of figure 1 are representative samples from these media. The difference in shape is not significant. The osmotic value of the simple solution of dextrose was of course nearly like that of the full nutrient medium, while distilled water with yeast extract alone had a much lower osmotic value.

Distilled water alone was apparently neither better nor worse for these root tips than was distilled water with addition of dextrose or of yeast extract. Some tests with tap water without any addition gave results like those obtained with distilled water either alone or with addition of dextrose or yeast extract.

It appears that neither dextrose alone nor yeast extract alone (with its uncertain but surely small content of mineral salts and organic material) could supply the requirements for growth of these excised tips, even for a short time. Addition of dextrose or of yeast extract to distilled water did not overcome the inadequacy of the water, although the food value of dextrose cannot be questioned and although the dextrose solution had almost the same osmotic value as the full nutrient medium, which gave pronounced growth. It may be supposed that dextrose not only supplied the carbohydrate needed for cell formation and extension but also furnished a requisite source of energy through respiration, and that inorganic nutrition was at first possible through salts originally present in the root tips. Some inorganic and also some organic material was doubtless derived from the yeast extract. Apparently the internal mineral supply and the salts derived from yeast extract were not adequate to maintain growth beyond the first few days. That rather good growth was obtained when the salts of the USPENSKI formula and dextrose were both externally supplied indicates, as might be expected, that these root tips required, for maintained growth, a continuous external supply of both carbohydrate and salts. When both dextrose and salts were present in the medium, growth was greatly accelerated by yeast extract, presumably because of the pres-

ence of some very active material derived from yeast or made available through the action of something derived from yeast.

That growth in the first two weeks of culture was nearly doubled when yeast extract was added to an already good solution is of special interest. It will be recalled that this effect was produced by extract from only 0.1 gm. of dry yeast per liter of solution. ROBBINS (**5**) tested a similar yeast extract and concluded that it was *not* beneficial to the growth of his maize root tips in the first two weeks after excision. However, he observed a stimulative effect due to his yeast extract, but that effect was manifest only after about six weeks of culture. The experiments of ROBBINS are of course not directly comparable with those reported here, for he was studying maize and he employed much larger pieces of root (mostly 10–60 mm. long) than were used in the present study.

Relations between growth and H-ion concentration of the medium received some attention. A series of cultures included sixteen different solutions, each represented by eight different initial pH values, as follows: 5.0, 6.0, 6.4, 6.8, 7.2, 7.6, 8.0, and 9.0. Adjustments of H-ion concentration were made with 0.01 M HCl or 0.2 M NaOH and pH values were measured with a potentiometer and Youden quinhydrone electrode, also being verified occasionally by use of the glass electrode. No attempt was made to buffer the solutions, since phosphate or borate buffers would have altered the concentrations of the constituent inorganic ions while citrate, phthalate, or glycine buffers would not only have affected the ion concentrations but would also have added organic constituents which might have influenced growth. This consideration, which seems to have been largely ignored in many studies on H-ion concentration, requires attention because it is difficult or impossible to distinguish between buffer action itself and other effects that might possibly be related to the buffer substances.

There were three cultures for each initial pH value of each solution and, in addition, a single blank flask (without root tip) for each solution as it was before adjustment. All flasks and their contents were autoclaved initially, but no H-ion measurements were made after autoclaving until the end of the culture period, after the root tips had been removed from the culture flasks. Consequently no information is available concerning the actual pH values when the roots were introduced into the solutions, and it is possible that hydrolysis, or other alteration of the solutions brought about by autoclaving, may have been somewhat different for originally different pH values of the same solution, and that pH values when the root tips were introduced may have been somewhat different from the corresponding values before autoclaving.

In general there was much apparently irregular variability in this series and most of the results may be omitted here, but three solution types are

considered briefly. They are designated as A, B, and C. These differed only with respect to salt content and salt proportions. All three were alike in that they contained per liter 20 gm. of dextrose and the extract from 0.1 gm. of yeast.

Solution A was like the full nutrient medium except that KH_2PO_4 (25 mg. per liter) was replaced by KCl (14 mg. per liter). The final pH values for the eight different initial H-ion concentrations of this solution were all nearly alike, and low (about 5.1), although the initial values (before autoclaving) ranged from 5.0 to 9.0, as has been noted. Autoclaving and standing for two weeks in contact with a root resulted, therefore, in altering the initial pH value only a little if it was low, but producing increasingly great reduction as the initial value was higher. An initial value of 9.0 became 5.3 at the end of the experiment. Such alteration might be expected, for this solution contained no phosphate (unless a trace of PO_4-ion may have been introduced as impurities or in the yeast extract) and the salts used have but little buffer action. The growth indices varied irregularly and no consistent relation was apparent between growth of the root tips and either initial or final pH values. Nevertheless all but the initially most alkaline variant of this solution (pH = 9.0) gave good growth.

Solution B was the full nutrient medium already referred to. As in the case of solution A, the final pH values were all low but they were consistently somewhat higher as the initial value (before autoclaving) was higher. For the initial series of values specified, from 5.0 to 9.0, the final values ranged in an almost linear series from 5.0 to 5.65. The differences in H-ion concentration were thus maintained in their directions throughout the 2-week period, although their magnitudes were generally very greatly decreased. The pH value that was initially lowest did not alter, but all the other initial values became very much lowered, more so as their magnitudes were greater. In this connection it is to be noted that solution B contained a considerable concentration of phosphate, an effective buffer, which was absent or present in only exceedingly low concentrations in solution A. The growth indices for the eight different initial pH values of solution B showed irregular variations, but these were not so pronounced as in the former instance. The lowest index value (41 mm.) was for the culture whose solution had the highest initial and final pH value, but all the other growth indices were without consistent relation to the pH value.

Solution C differed from solutions A and B (the full nutrient medium) only with regard to its phosphate concentration; it contained twice as much KH_2PO_4 per liter as is called for by the USPENSKI formula, and it was consequently more effectively buffered. Its final pH values were regularly lower than the corresponding initial values, as in the cases of solutions

A and B, the lowering being more pronounced as the initial values were higher. For initial values from 5.0 to 7.2 inclusive the final values formed a nearly linear ascending series from 4.8 to 5.9, but for initial values from 7.2 to 9.0 inclusive the final values were sensibly alike, about 5.95. Although there was considerable irregular variation in growth-index values, a rather consistent relation between growth and H-ion concentration is suggested by the numerical data for this series, which are as follows:

Initial pH values	5.0	6.0	6.4	6.8	7.2	7.6	8.0	9.0
Final pH values	4.8	5.0	5.3	5.4	6.0	5.6	6.0	5.95
Final growth-index values (mm.)	140.0	153.3	119.3	144.3	67.7	47.3	49.3	35.0

The growth index may be taken as about the same (from 119.3 to 153.3 mm.) for final pH values from 4.8 to 5.4, while it tended to be progressively somewhat smaller for initial pH values from 7.2 to 9.0. As has been said, the initial pH values refer to the solutions before they were autoclaved, and no information is at hand to show whether either the initial or final pH values really represent the environments of the root tips, or whether the initial or the final pH values were more effective during a larger part of the experimental period, etc. It is consequently not desirable to attempt any closer analysis of these relationships. An equilibrium value about 5.0–5.5 may be regarded as optimal for growth of these wheat root tips in the full nutrient solution (USPENSKI solution plus dextrose and yeast extract) and under the other conditions of this study. This represents a much greater acidity than has usually been maintained for cultures of animal tissues.

The three control solutions that were left unadjusted as to H-ion concentration and stood for two weeks without root tips showed final pH values of 5.1 (solution A), 4.8 (solution B), and 5.2 (solution C). In another experiment five flasks of a solution containing the usual proportions of salts and dextrose but no yeast extract were allowed to stand two weeks without root tips. They gave a mean final pH value of 5.55 ± 0.25. ROBBINS found that his culture solutions generally showed a final pH value about 5.4, and suggested that H-ion adjustment during the experimental period was brought about through some sort of buffer action due to root tips or yeast extract. This was obviously not the case in the control tests of the present study. ROBBINS does not give final pH values for any solutions that were allowed to stand throughout the experimental period without root tips or without root tips and yeast extract.

The influence of temperature on growth of these root tips of wheat seedlings in the full nutrient medium was studied by means of two experimental series, both conducted in darkness. In the first of these, six different, maintained temperatures (15°, 20°, 25°, 30°, 35°, 40°) were tested. There

were nine cultures for each temperature. At the end of the 2-week period a marked influence of temperature was indicated, as is shown by the representative roots depicted in figure 2. For 15° the growth index was 15 mm.;

FIG. 2. Representative excised root tips grown two weeks in full nutrient medium (salts, dextrose, and yeast extract in redistilled water) and in darkness, at six different maintained temperatures. About natural size. Temperatures represented are, reading from left to right: 15°, 20°, 25°, 30°, 35°, and 40°.

the tips appeared healthy but had very few and very short branches. For 20° the condition of the roots was about the same as for 15° but the growth index was 23 mm. For 25° the condition was excellent and the growth index was 62.5 mm., almost three times as great as for 20°; on the average there were seven branches per root tip. For 30°, on the other hand, the roots were evidently unhealthy; the growth index was only 8.2 mm. and there was only one branch per root tip on the average. For 35° and 40° the tips were blackened and swollen; no branches were present and no elongation had occurred. The optimal temperature appears to have been between 20° and 30°.

The second temperature experiment included maintained temperatures of 20°, 22°, 24°, 25°, 26°, 27°, 28°, and 30°, for which the growth indices

FIG. 3. Same as figure 2, but the maintained temperatures represented are, reading from left to right: 20°, 22°, 24°, 25°, 26°, 27°, 28°, and 30°. About ¾ natural size.

were respectively 81, 98, 89, 75, 74, 59, 91, and 33 mm. Representative root tips from this series are shown in figure 3. The highest temperature was obviously supra-optimal but no optimal temperature is clearly indicated. Nevertheless the appearance of the root tips and the manner of their growth indicated that the optimal temperature was about 26°–28°.

The influence of illumination on the growth of their excised maize root tips was considered briefly by ROBBINS and MANEVAL (7), who concluded that light was beneficial; but it was not clearly shown that temperature differences between lighted and unlighted flasks may not have been more or less effective in their experimental tests. To distinguish between light effects and those of heat is very difficult indeed. In the present study of excised wheat root tips some comparatively simple experiments were devoted to the influence of illumination on growth. For cultures in darkness each flask was covered with an opaque paper wrapper, black within but white on the outside. The cultures were distributed uniformly on a table, each row of covered flasks being accompanied by an adjacent row of uncovered ones.

One experiment comprised sixteen different solutions, with ten darkened and ten lighted cultures for each solution. The light supplied was ordinary diffuse daylight. All cultures were in darkness throughout the nocturnal part of each day, while the root tips in uncovered flasks received natural and fluctuating weak light during the diurnal part of each day. Those in covered flasks were in darkness throughout the entire 2 weeks of the experiment. It was presumed that all cultures had nearly the same temperature, about 22°. Cultures with solutions that were well suited to the growth of these root tips showed significantly more growth in the uncovered flasks than in the covered ones. With solutions that were not well suited to growth, however, this apparent influence of light failed to appear or was so slight as to be insignificant.

Another experiment on the influence of illumination comprised one hundred cultures in the full nutrient medium, fifty in covered flasks, and fifty in uncovered ones. The flasks stood on a table in a room provided with fair temperature control (about 25°) and with continuous electric light. Three 150-watt nitrogen-filled Mazda "daylight" bulbs with reflectors furnished illumination; they were 70 cm. above the plane of the table, about 75 cm. apart, and in a straight row, being so placed as to illumine the entire series about equally. Three covered and three uncovered flasks, each of which bore a thermometer whose bulb was immersed in a volume of water equal to the volume of solution regularly used, gave evidence concerning the culture temperatures. These were arranged in pairs at the left, center, and right of the group of cultures. The approximate mean temperature of the

water in the uncovered thermometer flasks for the 2-week period was 25.0°, while the corresponding mean temperature of the water in the covered ones was 0.7° lower. If it be supposed that these temperature values properly represent the temperature of the solutions in the two sets of culture flasks, it may be said that the illuminated root tips were kept generally about 0.7° warmer than those without light.

The average growth index for the fifty darkened cultures was 103.0 mm. while the corresponding value for the fifty illuminated (and presumably somewhat warmer) cultures was 120.2 mm. This is surely a significant difference; it amounts to 16.5 per cent. on the basis of the lower value. Furthermore, a similar difference was clearly indicated by ocular evidence; the darkened root tips were more slender and more sparsely branched than the others and they appeared less healthy; those grown with illumination were less slender, more richly branched, and apparently in better general condition, although somewhat more bent. In figure 4 a representative sample of the dark-grown roots is shown at the left and a representative sample of the others is shown at the right.

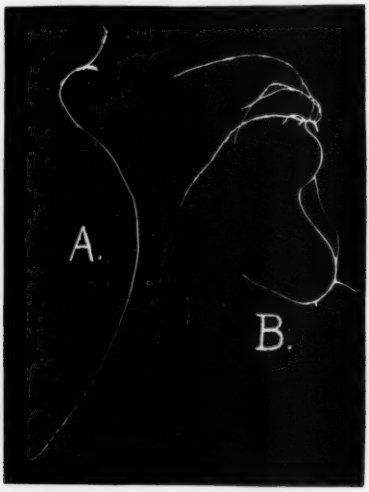

FIG. 4. Representative excised root tips, approximately natural size, grown two weeks in full nutrient medium: *A*, in darkness at about 24.3°; *B*, with continuous electric illumination, presumably at a temperature a little higher than that of the cultures in darkness.

Although a temperature difference of the order of 0.7°, maintained for two weeks, is not by any means to be regarded as negligible, yet it may be recalled that the experiments on temperature influence (see preceding dis-

cussion) gave no indication of great sensitiveness in these root tips to slight differences in temperature in the region of 24°–26°, and it consequently seems unlikely that the pronounced growth difference was mainly due to unlike temperatures of the darkened and illuminated solutions. It may therefore be said that these cultures of excised wheat roots showed a marked beneficial or stimulating effect due to such illumination as was tested. This conclusion supports the indications derived from the preceding experiment on illumination. ROBBINS and MANEVAL (**6**) came to a similar conclusion with regard to their excised maize root tips. They reported also that anthocyan was formed in their illuminated maize roots, but no coloring was observed in the cultures here described.

The degree of aeration of the culture solution might exert an influence on the rate of growth of these wheat root tips if the external supply of oxygen were a limiting condition. The general question of aeration was discussed to some extent by ROBBINS and MANEVAL (**7**).

Some experiments on aeration were carried out in the present study and their results remain to be presented. No elaborate procedures for securing different degrees of aeration of the medium were attempted, partly because it seems best to begin to approach new problems by means of relatively simple methods and partly because time and facilities for adequate control of oxygen supply and the disposal of CO_2, etc., were not readily available. The experiments of ZIMMERMANN (**13**) gave rise to the suggestion that depth of culture solution might serve as a rough control of oxygen concentration in the immediate vicinity of the root tip, and several different depths of solution were accordingly tested. The flasks used were all of the same size, but some had 50 ml. of solution, others had 25 ml., and still others had 10 ml. The three resulting depths of solution were approximately 20, 10, and 4 mm. respectively. All three groups exposed about the same area of solution to the air above (approximately 28 sq. cm.). Test-tubes containing 10 ml. of solution were also used, with liquid columns about 6 cm. high and an air-liquid area of about 2.5 sq. cm. Because the root tips generally lay at the bottom of the flasks, beneath the solution,[1] all oxygen reaching them from the air had to penetrate the solution either by solute diffusion or by convection, or by both means. To secure in this way different depths of solution in flasks of the same size naturally involved the introduction of another experimental variable, namely, the volume of medium present. But the test-tube and the flask containing the least volume of medium were com-

[1] This was not always true. Although the root tips regularly sank to the bottom of the flasks immediately upon being introduced, yet in many instances rapidly growing tips subsequently rose to the surface and remained there. No difference was observed, in either manner or rate of growth, between sunken and floating root tips. This constitutes indirect evidence that aeration was adequate, even at the bottom of the flasks.

parable with respect to depth and area, for the volume of solution used was 10 ml. in both instances. For these aeration tests the full nutrient medium was used; that is, the USPENSKI solution plus dextrose and yeast extract. Flasks and test-tubes stood in a room with temperature fluctuating approximately between 21° and 23°, with continuous artificial illumination provided by five 150-watt Mazda "daylight" lamps arranged as in the illumination tests already described.

The average growth indices for the four groups of containers, ascertained at the end of the 2-week experimental period, were as follows:

FLASKS:	Greatest depth,	50 ml. of sol.,	44.2 mm.
	Medium depth,	25 ml. of sol.,	41.3 mm.
	Least depth,	10 ml. of sol.,	32.3 mm.
TEST-TUBES:		10 ml. of sol.,	32.8 mm.

Aeration of the root tips was apparently adequate in both the flasks containing the medium volume of solution and in those containing the largest volume. This seems to imply that oxygen supply was more than adequate in the latter, which had the same volume as those regularly used throughout this study. The significantly smaller growth indices for the flasks that contained the smallest amount of solution is clearly not to be considered as due to excessive aeration; root tips at the bottoms of the test-tubes must have had less thorough aeration than even those at the bottoms of the flasks that contained the largest volume of solution, and the tubes gave growth just as satisfactory as that shown by the flasks that contained the smallest volume. The low (and nearly equal) growth indices for test-tubes and for smallest-volume flasks are apparently not related to aeration conditions at all. They may have been due to inadequacy in the volume of solution in these instances; as though the 10 ml. of the solution used did not contain a sufficient supply of some substance or substances necessary for the maintenance of growth for two weeks under the prevailing conditions. It is also logically possible, of course, that the retarding effect of inadequate volume of solution may have been due to accumulation of injurious material resulting from the metabolism of the root tip, material that might become more widely dispersed in the larger solution volumes. Doubtless the aeration and volume relations of such cultures as these are themselves influenced by the growth rates of the excised root tips, by the length of the experimental period, by the prevailing temperature, and by other current environmental conditions.

Optimal conditional complexes for growth of the excised wheat root tips of this study, in the 2-week period uniformly employed, embraced redistilled water, the salts and salt proportions of the USPENSKI solution, 20 gm.

of dextrose per liter of solution, yeast extract from 0.1 gm. of yeast for each liter of solution, a final or equilibrium H-ion concentration giving a pH value about 5.0, a maintained temperature of about 26°–27°, continuous electric light, and at least about 25 ml. of solution per culture of a single root tip. The numerical values from some cultures that gave remarkably extensive root systems under these conditions are given as follows:

Culture no.	I	II	III	IV	V	VI
Length of main root (mm.)	115	50	48	50	52	112
Total length of branch roots (mm.)	151	204	198	183	170	90
Growth index (mm.)	266	254	246	233	222	202

Culture no. 1 gave the greatest growth secured for any single root tip in any of the many cultures carried out under these conditions and for the 2-week experimental period. As is seen, the main root was 115 mm. long at the end of the period; there were five secondary roots totaling 133 mm. and a single tertiary root 18 mm. long. This represents a mean increment of root elongation of 19 mm. per day.

These experiments were carried out at the Boyce Thompson Institute for Plant Research in 1929–1930, while the writer held a National Research Council fellowship in botany. Laboratory facilities were kindly made available for this study by Dr. WILLIAM CROCKER, director of the Institute. The results were not assembled until the following year at the Pflanzenphysiologisches Institute of the University of Berlin, when the writer was a joint fellow of the National Research Council and the Rockefeller Foundation. In the course of the experimentation many helpful suggestions were received from Dr. L. O. KUNKEL, of the Boyce Thompson Institute, and much critical assistance in the final writing of this paper was received from Professor BURTON E. LIVINGSTON, of the Laboratory of Plant Physiology of the Johns Hopkins University.

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RÔLE OF "NUTRILITES" IN THE NUTRITION OF MOLDS AND OTHER FUNGI

ROGER J. WILLIAMS AND J. MARSHALL HONN

Introduction

Since the discovery by WILDIERS (**3**) of the substance (or substances) of vitamin-like nature, to which he gave the name "bios," much work has been done on the subject of yeast nutrition. Especially were investigations along this line stimulated by the suggestion of the senior writer (**5**) that bios might be identical with one of the vitamins. It has been amply demonstrated, however, that bios is not a single substance (**9**) and that different strains of *Saccharomyces cerevisiae* require traces of different unknown substances for their growth stimulation (**7**). Likewise, whereas the term vitamin has been restricted in its meaning to include only substances involved in animal nutrition, and there is abundant evidence to indicate that various bacteria, fungi, etc., have requirements of a vitamin-like nature, the broad term "nutrilite" (**6**) has been suggested to cover the vitamin-like substances which are involved in the nutrition of organisms in general.

It was the purpose of the work here reported to investigate whether the fungi other than yeast, which can be propagated in the laboratory, have requirements for, or are stimulated by, nutrilites or vitamin-like substances.

In an early experiment with yeast, one of us observed accidentally that a certain mold seemed to produce a yeast-growth stimulant. Ordinary yeasts themselves apparently produce yeast growth stimulants slowly, and hence are not so greatly affected by the presence of nutrilites when relatively large quantities of yeast are planted. It was to be expected, therefore, that in the case of molds the effect of nutrilites would be apparent only when the original seeding of the mold in a synthetic medium was light. The case of molds is somewhat complicated also by the fact that they grow from spores, and these spores would be expected to contain a certain amount of stored nutrilites which would affect the early growth of the fungus.

WILLAMAN (**4**) found that *Sclerotinia cinerea*, the brown rot fungus of fruits, did not develop to maturity properly unless a fruit juice was added to the synthetic medium in which it was planted, and concluded that the addition was due to a vitamin-like substance. LEPESCHKIN (**1**) reported that vitamin B had some effect on the growth of *Penicillium glaucum* during the early stages. Aside from these cases, no work has been found dealing specifically with the requirements of molds for what we choose to call nutrilites.

In selecting the molds to be used, an attempt was made not only to include those of the more common varieties, but also to choose species that were representative of the different types, Eumycetes (*Penicillia* and *Aspergilli*) and Phycomycetes. In the preliminary work, various wild molds were obtained by exposing some of the control media to the laboratory air, and from other sources. All but two of these were discarded.

The ten different species of molds and three pathogenic yeasts used during the experiments are listed here and the source of each given. The numbers preceding the names designate the cultures from the sources indicated. Two additional molds, *Sclerotinia cinerea* and *Rhizopus nigricans*, from the American type culture collection were not cultured successfully.

1. Blue-green mold (wild)
2. Light brown mold (wild)
3. *Penicillium roqueforti* (Roquefort cheese)

1015. *Aspergillus niger*
1019. *Aspergillus glaucus*
1117. *Penicillium expansum*
1216. *Mucor racemosis*
} American type culture collection

Pathogenic molds:

538. *Penicillium crevicaule*
989. *Microsporon fulvum*
2058. *Cephalasporium*
} Dr. Fred Weidman, University of Pennsylvania

Pathogenic yeasts:

1871. *Monilia penoyi*
1872. *Monilia metalondinensis*
1883. *Monilia macedoniensis*
} Dr. Fred Weidman, University of Pennsylvania

All of these molds and yeasts were grown on the standard molasses agar medium (**10**) which has been used in this laboratory for a number of years for the cultivation of stock yeast cultures.

In order to carry out the experiments and answer the question just outlined, it was desirable to have a synthetic medium which contained all the substances which molds are known to require except those of a vitamin-like character. The medium used throughout these experiments (designated as control medium) had the following composition and was a modification of Czapek's medium, in that the ammonium-ion was added (**2**) in the form of phosphate, and small amounts of manganese and zinc were added because they have been found to be necessary for some fungi.

	Grams
Sodium nitrate, $NaNO_3$	3.0
Potassium phosphate, K_2HPO_4	0.50

Potassium chloride, KCl	0.50
Magnesium sulphate, $MgSO_4$	1.00
Ferrous sulphate, $FeSO_4$	0.015
Ammonium phosphate, $(NH_4)_2HPO_4$	0.50
Zinc sulphate, $ZnSO_4$	0.050
Manganese chloride, MnCl	0.010
Sucrose	30.0
Water	1000.0

For a source of nutrilites a solution containing 1.0 gm. per 500 cc. of an aqueous-alcohol extract of yeast (Northwestern) was used. One cc. of this solution was added to 10 cc. of the control medium in the following tests.

In order to be reasonably sure that the effect of the addition of this yeast extract was not due to the presence of ordinary nutrients in it (amino acids particularly), another "synthetic" solution containing a similar amount of known amino acids was prepared and used as an additional control. At the same time it was possible to ascertain the effect of this amino acid mixture alone on the growth of the fungi in question.

Equal amounts of the following pure amino acids were mixed and 1 cc. of a solution containing 0.1 gm. of mixed amino acids per 500 cc. was added to the control medium as indicated. (The amino nitrogen content of the yeast extract as determined by the method of Van Slyke was the same as that calculated for the amino acid solution.)

d-arginine monohydrochloride
l-proline
l-leucine
l-histidine dihydrochloride
d-lysine dihydrochloride
l-tryptophane

Experimentation

Erlenmeyer flasks of 50-cc. capacity, sterilized, and plugged with cotton were used as containers for the media. At each planting of the individual mold species, four flasks were employed into each of which were placed 10 cc. of the control medium. Into one flask containing 10 cc. of the control medium was placed 1 cc. of the amino acid solution, and the flask marked *A*. To a second flask containing 10 cc. of the control medium was added 1 cc. of yeast extract solution, and this was marked *Y*. Into a third flask was placed 1 cc. of each of the amino acid solutions and the yeast extract in addition to the control medium and this flask marked *YA*. The fourth flask contained only the control medium and was marked *C*. Thus for each planting four different media were used. A mold was planted in all of these

four at the same time, and the rate and amount of growth and degree of maturity were noted from time to time.

To secure equal seedings in all media, the following method was developed for planting the molds. With a sterilized wire loop some spores were scraped from the surface of a stock culture and were shaken with a little distilled water until an even suspension was made. Enough spores were used to make the suspension cloudy, but in no case was the number great enough to cause visible cloudiness in the medium being seeded when 1 cc. of the suspension was planted in each flask. The plantings at various times were not exactly equivalent, of course, but for any one planting, the numbers of spores placed in the different flasks were made as nearly equal as possible. Some specimens produced few or no spores. In this case it was necessary, in order to make an even seeding, to shake bits of mycelium vigorously in water until the pieces were very small and equally distributed throughout the liquid.

The seeded flasks were placed in an incubator and kept at a temperature of 27°–29° C. Under these conditions growth was found to be considerably faster than when the cultures were exposed to the variations of room temperature. Higher temperatures than these caused evaporation of water, which condensing on the side of the flasks and running down, flooded the surface of the mold.

Observations were made about every two days, since in most cases the growth was not rapid enough for distinct changes to be seen within 24 hours. For the first day or two appearances were generally the same in all flasks, as the spores were germinating.

Some difficulty was encountered in making satisfactory estimations of the development of the mold at any time. Although the volume of the mold often appeared great, the actual dry weight of that from one flask would never be more than a few milligrams, and in many cases would be too small to weigh. It was necessary therefore to make some sort of comparative observation on the volume of growth. In the system used for this, a number was assigned to the amount of growth in one flask and if there was a larger growth in another flask, a higher number was assigned to it. If in one medium the growth was twice that in another, the number given to the first would be twice that given to the second, and so on. With a little practice the comparative amount of growth made in the different media could be estimated satisfactorily.

Some of the molds grew to greater volume before producing spores than did others. If the production of spores may be considered as an indication of the stage of maturity, then the volume of growth and the degree of maturity did not run parallel in all cases. For this reason it was thought

desirable to maintain a separate record of the production of spores. The same system was used as in the record of growth.

The molds as a whole were found to behave rather erratically in their growth. A certain species might grow satisfactorily for several different plantings, and then after one particular planting it would suddenly die in the media when only a few days old. A new planting, however, would grow as before. It will be noted that during the third planting of no. 5, *Aspergillus glaucus*, the mold died in the *YA* medium. This did not happen to this mold in any of the other plantings. Such behavior might be due to bacterial contamination.

Tables I–IX contain the results of observations. The composition of the medium is indicated by the letters appended. *C* stands for control medium; *A* for medium to which amino acids have been added; *Y* for the medium to which yeast extract has been added; and *YA* indicates the medium which contained both yeast extract and amino acids in addition to the control medium. Owing probably to slight variations in the amounts planted and to the masking effect of stored food materials present in the spores, a single experiment often does not show striking results. However, the totals of the horizontal columns show the general behavior of the various molds in the different media.

The determinations of the amounts of growth of the yeasts (pathogenic) were made with a thermo-couple devised for use in measuring the turbidity of suspensions (**8**). The more turbid a suspension of the yeast, the smaller the galvanometer deflections. The galvanometer readings have been translated into milligrams of moist yeast per cubic centimeter.

The results of the tests are summarized as follows:

1. Growing as well in the control medium as when additions were made: blue-green mold (wild), *Penicillium roqueforti, P. crevicaule.*

2. Stimulated in growth by amino acids: light brown mold (wild), *Aspergillus niger, A. glaucus, Penicillium expansum, Mucor racemosis, Microsporon fulvum, Cephalasporium.*

3. Stimulated in growth by yeast extract: light brown mold (wild), *Aspergillus niger, Mucor racemosis, Microsporon fulvum, Cephalasporium, Monilia metalondinensis, M. macedoniensis.*

4. Yeast extract producing distinctly more growth stimulation than did the amino acids: light brown mold (wild), *Aspergillus niger* (?), *Mucor racemosis, Microsporon fulvum, Cephalasporium, Monilia metalondinensis, M. macedoniensis.*

5. Spore formation stimulated by amino acids: blue green mold (wild), light brown mold (wild), *Aspergillus niger, A. glaucus, Penicillium expansum.*

TABLE I

GROWTH AND SPORE FORMATION, BLUE-GREEN MOLD (WILD)

	Days																Total
	3	5	7	2	7	3	5	2	4	6	3	5	7	1	2	3	
Growth																	
C	...	2	5	1	5	2	5	1	3	3	4	6	7	2	2	2	50
A	1	4	4	1	5	2	3	1	3	5	4	5	6	2	2	2	50
Y	..	...	...	2	5	1	5	1	5	6	2	4	4	3	4	4	46
YA	1	3	...	2	×*	2	4	1	4	5	2	3	4	3	4	4	42
Spore formation																	
C	...	...	5	...	3	...	5	...	...	1	1	2	7	...	...	...	24
A	...	3	4	...	3	...	3	...	1	5	2	3	6	...	...	...	30
Y	...	...	...	...	5	...	5	...	5	6	1	1	2	...	...	...	25
YA	...	2	5	...	...	...	3	...	2	5	1	4	4	...	...	1	27

* × indicates death of mold.

TABLE II

GROWTH AND SPORE FORMATION, LIGHT BROWN MOLD (WILD)

	Days																			Total
	3	5	13	14	3	5	7	4	6	2	3	5	1	3	4	5	3	5	7	
Growth																				
C	...	1	1	1	1	1	2	1	1	1	2	3	1	2	2	2	3	4	4	33
A	...	1	2	2	1	3	3	1	2	2	2	3	1	4	4	4	5	6	8	54
Y	1	2	2	3	2	4	5	2	4	3	3	4	1	4	5	5	2	4	7	63
YA	1	3	6	8	2	4	6	2	2	4	4	5	1	4	4	5	4	7	6	78
Spore formation																				
C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1	2
A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	6	8
Y	...	...	...	...	...	...	...	...	...	...	...	2	...	...	...	...	...	1	1	4
YA	...	...	3	4	...	...	...	...	...	...	2	4	...	...	...	...	...	4	7	24

TABLE III

GROWTH AND SPORE FORMATION, PENICILLIUM ROQUEFORTI

	DAYS							TOTAL
	1	5	2	7	3	5	7	
GROWTH								
C	1	5	3	6	4	4	4	27
A	1	4	2	6	3	5	5	26
Y	1	2	1	6	2	4	4	20
YA	1	1	1	6	2	4	4	19
SPORE FORMATION								
C	...	4	...	4	...	2	3	13
A	...	2	...	4	...	4	5	15
Y	...	...	...	6	...	2	3	11
YA	...	...	...	5	...	2	4	11

TABLE IV

GROWTH AND SPORE FORMATION, ASPERGILLUS NIGER 1015

	DAYS								TOTAL
	3	5	2	2	6	1	2	4	
GROWTH									
C	5	5	4	2	2	1	1	2	22
A	5	6	4	2	2	2	2	4	27
Y	4	4	4	2	4	3	4	5	30
YA	5	6	4	2	4	4	5	6	36
SPORE FORMATION									
C	3	3	3	2	2	...	...	1	14
A	5	6	3	2	2	...	...	2	20
Y	2	2	2	2	4	...	...	4	16
YA	4	5	3	2	4	...	...	6	24

TABLE V

GROWTH AND SPORE FORMATION, ASPERGILLUS GLAUCUS 1019

	DAYS															TOTAL
	3	5	2	3	1	3	4	5	8	3	5	7	2	3	5	
GROWTH																
C	3	4	2	6	1	4	7	8	8	3	6	10	2	2	2	68
A	3	5	2	6	2	5	8	8	8	6	6	10	2	3	3	77
Y	2	3	3	5	3	2	4	4	8	4	6	10	3	4	5	66
YA	2	3	3	5	4	4	×*	×	×	4	4	6	4	4	6	49
SPORE FORMATION																
C	1	1	...	3	...	2	2	2	2	2	2	3	...	1	1	22
A	1	1	...	3	...	4	5	5	5	2	2	3	...	2	2	35
Y	2	2	...	2	...	...	5	5	5	...	...	2	1	2	2	28
YA	2	2	...	2	...	...	...	...	...	2	3	4	2	4	4	25

* × indicates death of mold.

TABLE VI

GROWTH AND SPORE FORMATION, PENICILLIUM EXPANSUM 1117

	DAYS																	TOTAL
	3	5	7	2	4	1	3	4	5	3	5	7	3	6	2	3	5	
GROWTH																		
C	2	3	4	3	8	1	3	4	4	3	2	×	2	2	2	2	×	46
A	3	3	5	3	8	2	4	4	4	2	2	4	3	4	3	3	×	58
Y	1	3	3	1	7	3	3	3	3	1	1	3	4	5	3	4	×	48
YA	1	3	3	1	4	4	3	3	3	1	2	6	6	8	3	4	×	55
SPORE FORMATION																		
C	...	2	3	...	8	...	3	4	4	...	...	...	...	...	...	...	...	24
A	...	3	5	...	7	...	4	5	5	...	...	...	...	...	...	...	...	29
Y	...	1	1	...	4	...	1	3	3	...	...	...	...	1	...	...	...	14
YA	...	1	1	...	1	...	...	...	...	...	...	...	4	6	...	...	...	13

TABLE VII

GROWTH AND SPORE FORMATION, MUCOR RACEMOSIS 1216

	DAYS						TOTAL
	1	2	3	3	6	3	
GROWTH							
C	1	1	2	2	2	2	10
A	2	2	4	2	2	3	15
Y	3	3	4	4	5	4	23
YA	4	4	4	3	4	3	22
SPORE FORMATION							
C	...	...	...	1	2	1	4
A	...	...	...	...	...	1	1
Y	...	...	...	2	4	3	9
YA	...	...	...	...	3	2	5

TABLE VIII

GROWTH OF THREE PATHOGENIC MOLDS

	DAYS													
	No. 538, PENICILLIUM CREVICAULE			No. 989, MICROSPORON FULVUM								No. 2058, CEPHALOSPORIUM			
	3	7		1	2	5	7	1	3	TOTAL	1	4	6	TOTAL
M	1	2	C	2	4	5	6	0	2	19	2	2	6	10
A	1	×*	A	2	4	5	6	2	4	23	3	4	6	13
Y	1	×	Y	3	5	6	6	2	5	27	4	6	6	16
YA	1	2	YA	4	5	7	7	2	6	31	4	6	6	16

* Indicates death of mold.

6. Spore formation stimulated by yeast extract: *Mucor racemosis.*

7. Growth inhibited by amino acids: none.

8. Growth inhibited by yeast extract: blue-green mold (wild), *Penicillium roqueforti.*

9. Spore formation inhibited by amino acids: *Mucor racemosis* (?).

10. Spore formation inhibited by yeast extract: *Penicillium expansum.*

11. Both amino acids and yeast extract necessary for spore formation: light brown wild mold (striking).

TABLE IX

GROWTH OF THREE PATHOGENIC YEASTS

	Hours			Total
	48	30	36	
No. 1872, Monilia metalondiensis				
C	0.78	0.67	0.52	1.97 mg. moist yeast per cc.
A	0.87	0.65	0.48	2.00
Y	1.35	0.72	0.90	2.97
YA	1.55	0.77	0.78	3.10
No. 1883, Monilia macedonienis				
C	0.26	0.30	0.26	0.82 mg. moist yeast per cc.
A	0.31	0.30	0.26	0.87
Y	0.60	0.72	0.50	1.82
YA	0.74	0.77	0.63	2.14

	No. 1871, Monilia penoyi			
	Days			
	2	4	2	4
C	2	×*	2	×
A	2	×	2	×
Y	2	×	2	×
YA	2	×	2	×

* Indicates death of yeast.

Discussion

It has been found that the molds are rather difficult to work with and rather erratic in their behavior. Mixing of one mold with another, presumably, because of the activity of "mites" which crawl through the cotton plugs carrying spores with them, is one difficulty that had to be met, in addition to the difficulty of keeping spores in general from becoming dusted into all utensils. Bacterial contamination may enter into some cases, although the usual bacteriological technique was used throughout in handling the organisms. Wherever a foreign organism appeared to be present, the culture was discarded.

In spite of the irregularities of behavior, however, there is evidence that a number of molds and two or three yeasts tested (*Monilias*) are stimulated in their growth by vitamin-like substances present in yeast ex-

tract. It will be noted that the physiological actions of the yeast extract and the amino acid solution are both definitely positive but that they are distinct. As might be expected, the formation of spores with their relatively high protein content is stimulated in a number of cases by the presence of amino acids which are apparently synthesized with difficulty by several molds. Yeast extract has comparatively little effect in this regard. In stimulating growth, however, yeast extract has a greater effect than the amino acid solution in the case of seven organisms. The striking case of the light brown wild mold is worthy of note, inasmuch as both yeast extract and amino acids together are necessary for appreciable spore formation.

There appear to be more or less specific poisonous substances in the yeast extract which inhibit the growth of certain molds. Such substances are not in the amino acid mixture. We have previously observed in studies on yeast the existence in certain extracts of poisons which are effective toward certain strains of yeast and not toward others (**9**).

It was interesting to note that some of the molds seemed to be able to adapt themselves to the medium on which they were growing. This was particularly noticeable in the case of the light brown (wild) mold. After successive cultivations in the control medium this mold grew in this medium better than previously. This made it necessary to grow all stock cultures on a standard medium, as indicated previously.

The situation with respect to molds and their relationship to various nutrients seems to be fairly clear. Apparently for carrying on the living activities of an individual fungus, certain chemical materials are indispensable. Some of these, such as the inorganic ions and carbohydrate (or substitute) material, must be supplied in the food and cannot be produced by the organism. Other of the necessary cell constituents can be built up with varying ease. In the case of some molds (*Penicillium roqueforti* for example), apparently the protein building stones are built up with great ease, since introduction of amino acids into the medium has no apparent effect on the growth of the mold. On the other hand, such molds as *Penicillium expansum* were definitely stimulated in their growth by amino acids, and in spore formation a number were helped by the presence of the protein building stones.

The fact that in certain cases amino acids stimulate growth or spore formation does *not* mean, however, that the fungi in question are wholly unable to synthesize the amino acids. It merely means that synthesis does not take place with greatest ease.

Likewise with the nutrilites or vitamin-like substances which are apparently also necessary cell constituents: the fact that they stimulate growth does not mean that they cannot be synthesized by the organism, but

rather that they cannot be synthesized with ease. Accordingly, when presented to the organism ready-made, growth is enhanced.

In spores undoubtedly the necessary cell constituents are all stored, and for a time the organism is able to grow on this stored material. Accordingly, the growth at first is apparently the same whether nutrilites and amino acids are furnished in the medium or not. It is during the second phase of growth that the effect of nutrilites becomes apparent in a number of cases. If left in the same medium for a long time, the mold eventually (though slowly) produces enough of the essential organic cell constituents so that the whole of the food material may be used and the final growth may be approximately the same, whether or not nutrilites were present in the medium. In some cases, where the nutrilites are synthesized by the organism with great difficulty, the growth may continue to be very slow. There is the possibility, already mentioned, however, that the organism may adapt itself to a medium and increase its synthetic powers.

That certain molds are capable of producing substances belonging to the category of nutrilites was confirmed in this laboratory in connection with this study, inasmuch as a culture of *Aspergillus niger* grown on a synthetic medium was extracted and found to contain the nutrilite or "bios" for WILDIERS' yeast.[1]

Summary

Ten molds and three yeasts (*Monilias*) were cultured in various media, and from the study the following principal conclusions are drawn:

1. Several molds are apparently stimulated in their growth by substances of vitamin-like character which we have called nutrilites.

2. A number of molds sporulated much more freely when growing in a medium containing a mixture of pure amino acids.

3. The effects of nutrilites (and amino acids) on mold growth are masked in part by the presence of stored materials in spores. Yeasts respond more readily to the presence of nutrilites in the medium than do molds.

4. The fact that growth is stimulated by the presence of certain indispensable substances does not mean that the organism is unable to synthesize them, but rather that synthesis takes place with difficulty and consequent slowness. Molds, in common with yeasts, are in general apparently able to synthesize nutrilites slowly.

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FURTHER NOTES ON THE NUTRIENT REQUIREMENTS AND THE HISTOLOGY OF THE CRANBERRY, WITH SPECIAL REFERENCE TO THE SOURCES OF NITROGEN[1]

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(WITH TWO FIGURES)

Introduction

A project concerned with the nutrient requirements and the histology of the cranberry (*Vaccinium macrocarpon* Ait.) has been in progress at the New Jersey Agricultural Experiment Station for two years. Preliminary experiments (**1**) showed that the cranberry plant is capable of utilizing ammonium nitrogen. After these experiments were initiated, TIEDJENS and ROBBINS (**12**) showed that for tomato and certain other crop plants either ammonium or nitrate was a favorable source of nitrogen, but that the ability of the plants to utilize these different sources of nitrogen varied with the hydrogen-ion concentration of the nutrient medium, nitrate being superior to ammonium in a decidedly acid nutrient solution (pH 4 or 5) whereas ammonium was superior to nitrate in a neutral or slightly alkaline solution (pH 7 and above). The present investigation was therefore undertaken with the special object of determining the ability of the cranberry plant to utilize different sources of nitrogen at various hydrogen-ion concentrations. The problem is of practical importance since cranberries are grown commercially in bog soils of high acidity.

Methods

CULTURAL

The plants selected for these experiments were of the Howe variety, chosen as preferable to the Early Black of previous experiments (**1**) because of their larger size and more vigorous growth.[2] In the fall of 1930 several hundred one-year-old rooted cuttings were selected for uniformity; the roots were washed carefully, the plants set in 8-inch pots of washed quartz sand, and the pots buried in sand in a cold frame and covered with straw and brush. Throughout the winter, water was applied at regular intervals. At the onset of warm weather in the spring, the potted plants were removed to a refrigeration room, where they were maintained at a temperature slightly below 0° C. for a month, until such time as it was practicable to start experiments in the greenhouse.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station.

[2] The plants were obtained through the courtesy of Miss ELIZABETH C. WHITE, of Whitesbog, N. J.

On June 22, 1931, most of the plants were transferred to 3-gallon glazed porcelain crocks filled with washed quartz sand, five or six plants to a crock. They were then divided into three series, which until the latter part of August were kept in the greenhouse and received the following nutrient treatments.

SERIES 1.—Fifteen crocks received a complete nutrient solution containing nitrogen in the form of calcium nitrate. The solution R_2S_2 of previous experiments (**1**) was selected because it has been shown to support vigorous vegetative growth.

SERIES 2.—Fifteen crocks received a complete nutrient solution containing nitrogen in the form of ammonium sulphate. This solution also had been shown to support vigorous vegetative growth.

SERIES 3.—Fifteen crocks received nitrogen in the form of glycine. Calcium nitrate of the solution of series 1 was replaced by calcium chloride and an equivalent amount of nitrogen was supplied as glycine.

The composition of the various solutions is shown in table I. All had

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS USED

SERIES	SALT	PARTIAL VOLUME-MOLECULAR CONCENTRATION
Nitrate	KH_2PO_4	0.0049
	$Ca(NO_3)_2$	0.0049
	$MgSO_4$	0.0099
Ammonium	KH_2PO_4	0.0020
	$CaCl_2$	0.0073
	$(NH_4)_2SO_4$	0.0014
	$MgSO_4$	0.0071
Glycine	KH_2PO_4	0.0049
	$CaCl_2$	0.0049
	$MgSO_4$	0.0099
	$CH_2 \cdot NH_2 \cdot COOH$	0.0032

a total osmotic concentration of approximately one atmosphere. Iron was supplied from time to time as ferrous sulphate. In each series the fifteen crocks were divided into three groups, which received the nutrient solution at an initial hydrogen-ion concentration of 4, 6, and 8 respectively, the

reaction being adjusted by the addition of potassium hydroxide or sulphuric acid. Each crock received daily 1 liter of nutrient solution, which for the pH 4 and 6 cultures was supplied by SHIVE's constant-drip method (**11**), and for the pH 8 cultures (because of precipitation) was mixed in a jar and applied twice daily. All of the cultures were flushed with distilled water once a week.

In addition to the three series of sand cultures, two series of soil cultures were set up. Plants similar to those of the sand cultures were transplanted to bog soil in 8-inch clay pots. Nine pots received nitrogen in the form of calcium nitrate and nine in the form of ammonium sulphate. To three pots of the nitrate series and to three of the ammonium series were added 80 grams of lime, to three were added 40 grams and to three no lime was added.

HISTOLOGICAL AND MICROCHEMICAL

Plants from the cultures just described furnished material not only for study of general growth responses, but also for microscopic examination. Most of the microchemical tests employed were suggested by ECKERSON (**2**) of the Boyce Thompson Institute. For both histological and microchemical work fresh material was employed; although the endophytic fungus associated with the growth of the cranberry plant (**10**) is extremely small, it can be observed in fresh material, especially if stained lightly with ruthenium red, cotton blue, or mercurochrome.

Results

CULTURAL

In none of this season's sand cultures did linear growth of stems equal that of the previous season (**1**), for the cuttings were transferred to sand cultures and supplied with nutrient solutions much later; and prior to being transferred they were in a very inactive state, with the result that they were slow in resuming rapid growth. However, all of the plants of this season's series were on a par when they were set out, and within a few days all had started to produce new stem growth. Thus the comparative rates of growth in the different cultures are of significance. All of the plants produced some increase in stem length, although in the $\frac{\text{glycine}}{\text{pH 8}}$ and the $\frac{\text{nitrate}}{\text{pH 8}}$ cultures the increase was very slight, being limited to the initial growth just mentioned. By the middle of July decided differences were noticeable. The $\frac{\text{glycine}}{\text{pH 8}}$ and the $\frac{\text{nitrate}}{\text{pH 8}}$ plants showed a chlorosis from which they failed to recover upon the addition of either iron or boron. By

July 30 the $\frac{\text{glycine}}{\text{pH 8}}$ plants were so nearly dead that they were discarded, and the $\frac{\text{nitrate}}{\text{pH 8}}$ plants followed on August 6.

Of the nitrate cultures that persisted after August 6, all showed less growth than either the ammonium or the glycine cultures. There was little difference between those at pH 4 and those at pH 6; all were dark green and apparently healthy, those at pH 4 slightly more so than those at pH 6, but none was vigorously vegetative. When linear growth of stems was measured on August 24, there was very little difference between them. These results, as well as those in the other sand cultures, are shown in table II.

TABLE II

LINEAR GROWTH OF STEMS IN SAND CULTURES, JUNE 27 TO AUGUST 24

CULTURE	LINEAR GROWTH OF STEMS (AVERAGE OF 25 PLANTS) IN CM. PER PLANT
	cm.
Ammonium, pH 4	36
Ammonium, pH 6	88
Ammonium, pH 8	81
Nitrate, pH 4	34
Nitrate, pH 6	30
Glycine, pH 4	86
Glycine, pH 6	69

The glycine plants at pH 4 and pH 6 grew well, as did the ammonium plants at pH 6 and pH 8. All of these plants were dark green, produced new stem growth, had apparently healthy root systems, and presented an appearance that in all respects was satisfactory to a commercial cranberry grower. Over a period of several weeks the $\frac{\text{glycine}}{\text{pH 4}}$ plants were superior in appearance to any of the other plants in sand culture, although by August 24 little or no difference was evident between them and the ammonium plants at either pH 6 or pH 8. The $\frac{\text{ammonium}}{\text{pH 4}}$ plants remained stunted, and ultimately showed an injury to the stem tip that the addition of manganese and boron did not alleviate; they were discarded on August 26.

On August 27, stem tips and leaves from the most vigorously growing sand culture of each series were analyzed for alpha-amino nitrogen and for ammonia. The results, computed on a green weight basis, are as follows:

PLANTS	α-AMINO N	AMMONIA
ammonium / pH 8	0.031%	0.0053%
glycine / pH 4	0.040	None
nitrate / pH 4	0.017	None

Figure 1 shows the various cultures as they appeared on August 5. As stated, many of the cultures produced a considerable amount of new stem growth after that date.

Of the soil series, several cultures produced excellent stem growth and dark green leaves. Of these cultures the ammonium plants that had been supplied with a medium quantity of lime stood first, both in general appearance and in amount of linear growth of stems. Several of the plants retained after the conclusion of the experiments were growing vigorously at the end of six months. Second in amount of linear growth were the ammonium plants that had received no lime and the nitrate plants that had received a medium amount of lime. The others, ammonium plants receiving a large quantity of lime and nitrate plants with medium and high quantities of lime, were far less vigorous in growth, although they were not markedly chlorotic and during the course of the experiment it was not deemed necessary to discard them.

To furnish supplementary evidence, plants several years old were collected from bogs and brought to the laboratory for chemical analysis. All were of the variety Early Black, but they differed decidedly in vegetative vigor, according to the nutrient conditions of the bogs in which they had been grown. Some plants were highly vegetative (although fruitful), with long succulent shoots and green leaves. Others were much less vigorous, with shorter and more woody shoots, and many of the leaves were red with anthocyanin pigments; microscopic examination showed a much larger proportion of thick-walled cells, a heavier cuticle, and larger deposits of oil. A third group seemed intermediate between the other two in regard to the degree of vegetative vigor, although more nearly like the weakly vegetative than the strongly vegetative group.

Leaf blades only were analyzed. Amounts of ether extract, on the basis of dry weight, were as follows:

Strongly vegetative plants	10.57	per cent.
Intermediate plants	12.42	" "
Weakly vegetative plants	14.58	" "

Histological and microchemical

A previous paper (**1**) describes the stem and root structure of the cranberry plant, and includes some discussion of the distribution of the endophytic fungus in these organs.

In this season's work, the endophytic fungus was found in all plants of both sand and soil cultures, both in association with the roots and distributed through the entire stem system. A decided difference in amount of mycelium was noticed, however, between the rapidly growing plants and those that were stunted and chlorotic. In all of the rapidly growing plants, such as those grown with $\frac{\text{glycine}}{\text{pH 4}}$, the cells of the stem tip, even embryonic cells, were crowded with mycelium. Slightly farther back the mycelium was especially evident in pith, although it could be observed in all types of cells, including the epidermis. Many of the hyphae had swollen tips (fig. 2), which did not stain with either ruthenium red or cotton blue, but which showed a high content of protein and a few oil globules. Tests with gold chloride and with iodine-ammonia indicated an appreciable content of tannin in these swollen tips as well as in the cells of the cranberry stem. Janse (**4**) figures apparently similar structures in the mycorrhiza of certain Javanese plants. In the more mature portions of the stem, mycelium was observed in various stages of disintegration, as previously described (**10, 1**). Within these masses of disintegrating material there could often be observed conspicuous granules varying in color from brown or black to a deep cherry red. They were sometimes evident in cells in which strands of mycelium were still apparently intact.

On the other hand, the non-vigorous stunted plants, such as the $\frac{\text{nitrate}}{\text{pH 6}}$ ones, contained comparatively little mycelium at any time during the season's growth. Mycelium could be distinguished in the stem, but not abundantly, and most of it was in the granular stages of disintegration, even near the growing tip. Much the same condition had been noted in the dormant cuttings at the initiation of the experiments on June 22.

As in the plants of previous experiments (**1**), nitrates could not be detected at any time in either the fungus or the cranberry plants of any cultures. By means of the Nessler test and the formation of ammonium chloroplatinate crystals, ammonium could be detected in the tissues of the plants to which it had been supplied in the nutrient solution. It was especially evident within a few hours after a plant that had grown in a defi-

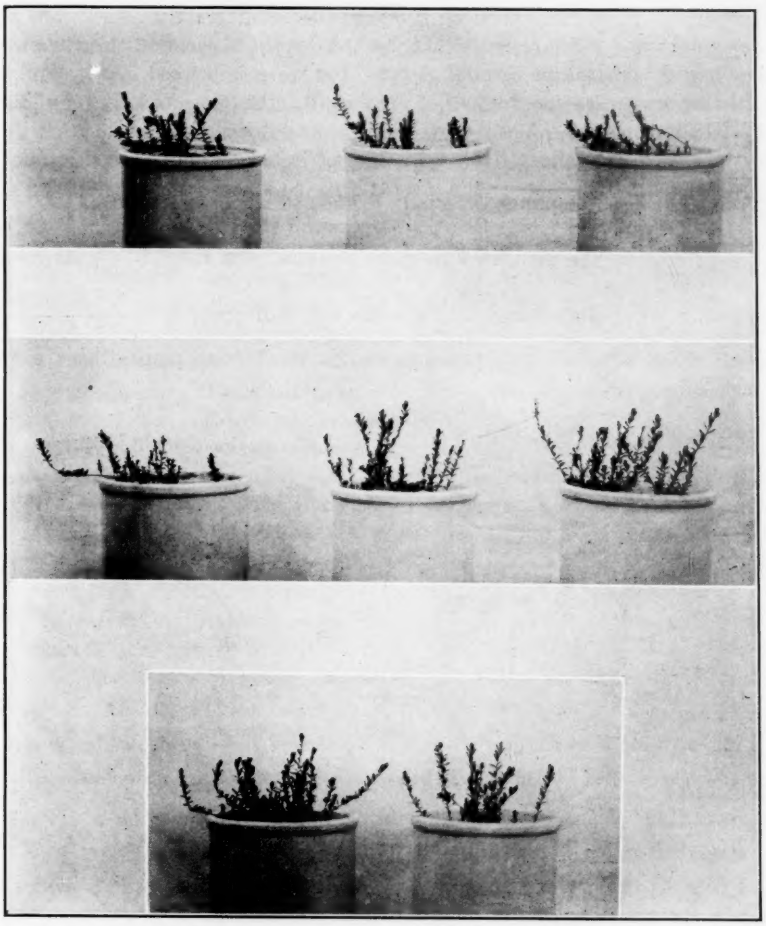

FIG. 1. Plants of sand cultures as they appeared on August 5, 1931: top (left to right), nitrate cultures at pH 4, 6, and 8 respectively; middle (left to right), ammonium cultures at pH 4, 6, and 8 respectively; bottom (left to right), glycine cultures at pH 4 and 6 respectively. $\times 1/11$.

ciency of nitrogen was transferred to an ammonium-containing nutrient solution.

Glycine was apparently present in the stems of plants to which it had been supplied in the nutrient solution. To fresh sections of young stem, absolute alcohol was added. Within an hour there appeared very small

crystals, which in the course of 12 hours increased greatly in size. With the exception of a few crystals that were obviously of some other substance, they had the crystalline form of glycine, and when immersed in oily liquids of the several refractive indices of glycine (**5**), the respective facets of the crystals disappeared. These facts would seem substantial evidence that the crystals were of glycine. They were present in large numbers in the young stems of the $\frac{\text{glycine}}{\text{pH 4}}$ plants, especially in the outer cells of the pith. A few were present in the mycelium in these sections. In the $\frac{\text{glycine}}{\text{pH 6}}$ plants a somewhat lower concentration of glycine was indicated; in the $\frac{\text{ammonium}}{\text{pH 8}}$ plants a few crystals were present; and in the nitrate plants there were practically none.

FIG. 2. Cells from stem tip of strongly vegetative $\frac{\text{glycine}}{\text{pH 4}}$ plant, showing swollen tips of hyphae: *A*, as seen in cross-section of stem; *B*, as seen in longitudinal section. × 600.

Discussion

From the present experiments, as well as earlier work (**1**), it is evident that in the cranberry, as in many other plants that have been studied by other workers (**6, 7, 8, 9**), degree of vegetative vigor is associated with the nitrogen metabolism of the plant. In the cranberry, however, food is stored not only as starch but also as oil; in the leaves, starch seems to be transitory and most of the stored food is in the form of oil, whereas in the stem large deposits of starch may be found. Chemical analyses of bog plants show that there is a definite correlation between degree of vegetative vigor and percentage of fats and lipoids in leaf blades (page 648), the percentage of ether-soluble substances being lowest in the most vigorously vegetative plants.

The cultures of these experiments, both sand and soil, indicate that young cranberry plants are capable of utilizing a variety of different substances as sources of nitrogen, but that the ability to use different substances varies with the reaction of the nutrient solution. In sand cultures nitrates can be used to at least fair advantage in acid but not in alkaline reaction, while ammonium can be utilized in neutral or alkaline but not in highly acid reaction. These results are in general agreement with those of TIEDJENS and ROBBINS (**12**) for tomato and other plants, although apparently the cranberry can utilize ammonium in somewhat more acid reaction than could the plants with which they worked. The findings are interesting in relation to the fact that cranberries are grown commercially in bog soil that is decidedly acid, even more so than the pH 4 cultures of these experiments, and that the most commonly used nitrogenous fertilizer is sodium nitrate. It might be expected, then, that the bog soil cultures to which ammonium sulphate was added would be inferior to the nitrate pots. Quite the opposite was true, however, for the ammonium cultures, both with and without lime, produced stem growth exceeding that of any of the nitrate soil cultures. The most plausible explanation of the difference between soil and sand cultures lies in the greater buffer properties of the bog soil. The present experiments did not include the application of ammonium sulphate over a period of several years.

As stated, the ammonium ion seemed to be readily absorbed by the cranberry plant (or its endophyte), and could be detected in its tissues; whereas nitrates could not be detected in either the cranberry or its endophyte at any time, indicating that if nitrates are absorbed as such, they must be converted into other substances immediately. Moreover, bog soil is usually low in nitrates.

It is generally assumed that one of the first steps in the synthesis of proteins is the reduction of nitrates to nitrites and ammonia (**3**). If this be true, it would seem reasonable to suppose that under proper environmental conditions the ammonium ion should furnish a more readily available source of nitrogen than does the nitrate ion. Such a conclusion is borne out by experiment. It has been observed repeatedly in this laboratory that the addition of ammonium salts to plants low in nitrogen produces more rapid response than the addition of nitrates. One commercial grower, reporting the application of ammonium sulphate to a cranberry bog over a period of several years, states that at first it produced greater vegetative growth than either sodium nitrate or dried blood, and that the leaves were dark green; but that after ammonium sulphate had been applied for several years the foliage became noticeably lighter, there was a decrease in the amount of fruit produced, and the berries were of poor color and keeping

quality. It is apparent that the symptoms are those of an excess of nitrogenous fertilizer, resulting in excessively vigorous vegetative growth and ultimately in a decreased production of fruit. The cranberry seems to be more sensitive than the tomato and certain other plants to an oversupply of nitrogen. It should be remembered also that not only is ammonium probably a more quickly available form of nitrogen than is nitrate, but that the amount of nitrogen in a molecule of ammonium sulphate is approximately 25 per cent. more than in a molecule of sodium nitrate, necessitating the use of a smaller quantity of the salt as fertilizer.

Several of the plants that remained from sand-culture experiments reported in a previous paper (**1**), and that had been stored in a cold frame through the winter and spring, were removed to the greenhouse on July 22 and supplied with the nutrient solution containing ammonium sulphate as a source of nitrogen, at pH 6. New stem growth became evident within two days, and within a week there were many new shoots two inches or more in length. Throughout the remainder of the season (their second season with ammonium as the only source of nitrogen) growth was vigorous and apparently healthy. It would thus seem sure that for cranberry, as well as for tomato and other plants reported by TIEDJENS and ROBBINS (**12**), ammonium in itself is not toxic, but only demands a satisfactory reaction. Nitrates could not be detected in any of the ammonium drips at any time, thus apparently precluding the possibility of nitrification of the ammonium in the soil. Macrochemical analysis (page 647) showed a considerably higher concentration of alpha-amino nitrogen in the plants that had been supplied with ammonium than in those that had been supplied with nitrate. These results are in excellent agreement with those of TIEDJENS and ROBBINS (**12**) and suggest that the ammonium absorbed was rapidly converted into amino acids within the plants.

According to the generally accepted theories of protein synthesis, proteins are built up from amino acids. If, then, it were possible for a plant to absorb amino acids, they should be a readily available source of nitrogen. Preliminary experiments (**1**) suggested this to be true. The glycine series of the present experiments has yielded further evidence. Not only did the glycine cultures produce vegetative growth, but over a period of several weeks the $\frac{\text{glycine}}{\text{pH 4}}$ plants were superior in appearance to any of the other sand cultures, and when linear growth was measured on August 24 they were found to have produced practically as much stem growth as the best ammonium cultures, and more than any of the nitrate cultures.

It may be suggested that within the soil solution of these cultures glycine was transformed to ammonium, and that as ammonium it was absorbed by

the plant. To be sure, the drip from these cultures was found to contain ammonium, but only in extremely small quantities. Moreover, the plants were growing better with glycine as a source of nitrogen than with ammonium at the same hydrogen-ion concentration. Also a far greater concentration of glycine was detectable as crystals within the plant when glycine was present in the external medium than when it was not. It thus seems reasonably sure that the cranberry plants of these cultures were dependent upon glycine as a source of nitrogen, not upon substances that were formed from glycine.

How much of the plant's ability to utilize glycine is attributable to the activities of its endophytic fungus has not been determined. By way of comparison, several tomato plants were grown in sand culture supplied with nutrient solution containing nitrogen in the form of glycine, at pH 4, 6, and 8. Over a period of several weeks all of these plants grew with vigor and produced good fruit. Glycine was detected microchemically in both roots and stems. It would thus seem that green plants, irrespective of mycorrhiza, may under certain conditions be able to utilize amino acids as a source of nitrogen. It is possible that the success of dried blood as a fertilizer for cranberries lies in the fact that upon disintegration it may yield amino acids and ammonium.

In all of the vigorously growing sand cultures it was noticed that mycelium of the endophytic fungus was very conspicuous in the tissues, especially the parenchymatous tissues of the young stems, penetrating even the embryonic tip (page 648). In all of the less vigorous cultures, whether of the nitrate, ammonium, or glycine series, far less mycelium was evident, and most of it was in the granular stages of disintegration. Essentially similar results were noted in the minus-nitrogen series of earlier experiments (**1**). Considered collectively, these observations suggest that the amount of mycelium of the endophytic fungus in the stem tissues varies more or less directly with the vigor of vegetative growth.

The present experiments tend to substantiate a conclusion that has been gathering form for several years as a result of various pieces of work, namely, that if under two sets of environmental conditions plants are produced which in all visible respects resemble each other, these plants will not differ materially in histological structure or in content of elaborated materials. There may be minor differences, such as the concentration within the plant of certain mineral salts or their ions that are supplied in the soil solution. For example, in the $\frac{\text{glycine}}{\text{pH 4}}$ and $\frac{\text{glycine}}{\text{pH 6}}$ plants of the current experiments, more glycine can be detected than in the plants of the other series; also, ammonium can be detected in plants of the ammonium

series but not of the others. Except for such minor differences, it would be impossible to distinguish histologically or microchemically between the most vigorously growing cultures of the glycine and of the ammonium series. Both could easily be distinguished from the best cultures of the nitrate series by their more vigorous growth, as well as by histological differences; but the nitrate cultures would be found to differ little histologically or chemically from the less vigorous cultures of the ammonium series.

Summary

1. Young cranberry plants were grown for two months in sand cultures supplied with nutrient solutions containing nitrogen in the form of calcium nitrate, ammonium sulphate, and glycine respectively. Each of the three series received the nutrient solution at three different hydrogen-ion concentrations, *viz.* pH 4, 6, and 8.

2. The plants that received ammonium at pH 6 and pH 8, and those that received glycine at pH 4 and pH 6, grew most vigorously, producing long stem growth, dark green foliage, and apparently healthy root systems. The $\frac{\text{ammonium}}{\text{pH 4}}$ and the $\frac{\text{glycine}}{\text{pH 8}}$ plants were stunted and chlorotic.

3. The nitrate cultures producing the greatest stem growth were at pH 4. However, all of the nitrate cultures were inferior in vegetative growth to the glycine cultures at pH 4 and pH 6 and the ammonium cultures at pH 6 and pH 8.

4. It is evident from these experiments that the cranberry can utilize several different sources of nitrogen, including nitrate, ammonium, and glycine.

5. The results of the present experiments confirm the findings of TIEDJENS and ROBBINS with certain other plants, that in sand cultures nitrate can be utilized to best advantage in acid solutions, and ammonium in more nearly neutral or in alkaline solutions. Apparently the cranberry is capable of utilizing ammonium under slightly more acid conditions than are tomato and certain other plants. Of the reactions tried, glycine was most successful at pH 4.

6. Soil cultures indicate that in bog soil ammonium can be used at a somewhat higher hydrogen-ion concentration than in sand. The difference may be attributable to the greater buffer properties of the bog soil.

7. Tests indicate that in the glycine cultures of these experiments, glycine was absorbed as such.

8. Mycelium of the endophytic fungus was found throughout the stem tissues, especially in parenchyma and in the cells of embryonic tips. The

amount of mycelium appears to be directly correlated with the vigor of vegetative growth, being abundant in the growing tips of vigorous plants and less abundant and partially disintegrated in less vigorous plants.

9. Analysis of bog plants showed a correlation between degree of vegetative vigor and concentration of fats and lipoids in the leaves, the most vigorously vegetative plants having the lowest concentration of ether-soluble substances.

10. It is suggested that in the cranberry, as probably in other plants, there is little difference in histology and composition of elaborated materials between plants that have attained the same degree of vegetative vigor, although they may have been grown under different environmental conditions.

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RELATION OF WESTERN YELLOW PINE SEEDLINGS TO THE REACTION OF THE CULTURE SOLUTION

JOSEPH HOWELL, JR.

(WITH SEVEN FIGURES)

Introduction

The diversity of habitats under which western yellow pine (*Pinus ponderosa* Law.) grows throughout its extensive range indicates the ability of this species to adapt itself to many environmental conditions. SAMPSON (**34**) noted that the species grew best in acid soils and was stunted in alkaline soils, while BAKER (**4**) found that soil reaction had little effect on the development of its seedlings. In the forests of the eastern United States WHERRY (**41**) found that conifers had a predilection for the acid soils. BAKER and KORSTIAN (**5**) made an extensive study of the reaction of soils in the Great Basin, and found that the western yellow pine grew in soils of pH 7.0 to 9.0. The writer found that, in the Sierras, western yellow pine grows on soils of a reaction of pH 5.0 to 8.5. This gives a total known range of from pH 5.0 to 9.0, and, from all indications, does not cover the entire range of reaction over which the species can exist.

Accordingly, there were three objectives in this investigation, as follows: (1) To study the range of reaction (acidity and alkalinity) which the seedlings of western yellow pine can endure. (2) To observe the effects of the pH reaction on the growth and development of its seedlings. (3) To determine what effect the pH reaction has on the expressed sap of the seedlings.

Procedure

Investigators (**17, 37**) have found that soil and sand cultures are unreliable for studies of reaction, in that many other factors influence the results. The greater number of investigators (**7, 17, 18, 37**) have, therefore, resorted to water cultures, in which they were able to decrease the activity of many interfering factors. In this study water cultures were used in order to secure as nearly as possible an equality of conditions for plant growth. Table I lists the culture media used.

Two milliliters of 0.5 per cent. solution of ferric tartrate were added to the original culture solution, as well as a like portion each week. The molar solutions were made up separately, using C.P. salts and ordinary distilled water. The culture solution was made by diluting the required amounts of the molar solutions with ordinary distilled water. Care was taken in mixing the various solutions so as to prevent the precipitation of

TABLE I

COMPOSITION OF STOCK AND CULTURE SOLUTIONS

SALTS	MOLAR CONC. GRAMS PER LITER OF STOCK SOLUTION	CALIFORNIA SOLUTION; MOLAR SOLUTION PER LITER	IONS	P.P.M.	PH OF CULTURE
	gm.	*ml.*			
$Ca(NO_3)_2 \cdot 4H_2O$	236.2	3.9	Ca	157	5.2
			K	181	
KNO_3	101.1	3.6	Mg	55	
$MgSO_4 \cdot 7H_2O$	246.5	2.2	NO_3	709	
			SO_4	212	
KH_2PO_4	136.2	1.1	PO_4	105	

the phosphate ion. The ferric tartrate was added after the other solutions had been diluted and well mixed in order to prevent the precipitation of insoluble iron compounds.

Changes of solution were made every four weeks in order to prevent serious changes in the composition of the cultures. The pH values of the cultures were adjusted by the addition of measured quantities of standard sulphuric acid or of potassium hydroxide. The pH values were determined colorimetrically (**8**), with frequent checks with the hydrogen electrode. Examinations and adjustments were made daily because of the rapid changes in the reaction on the alkaline side of neutrality.

TABLE II

MAXIMUM CHANGES OF REACTION IN 24 HOURS

PH OF SOLUTION	MAXIMUM CHANGE	PH OF SOLUTION	MAXIMUM CHANGE
2.7	0.1	7.0	0.3
3.0	0.1	8.0	0.3
4.0	0.2	9.0	0.4
4.5	0.2	10.0	0.4
5.0	0.2	11.0	0.5
6.0	0.2		

Selected seeds of western yellow pine collected near Georgetown, California, germinated between sterile blotters, and selected germinated seeds were transferred to the water cultures. Stoneware jars of 14-liters' capacity were used as containers, and two such jars containing 35 seedlings constituted a set. The following pH values were used: pH 2.7, 3.0, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0. The plants were allowed to grow

for a period of 133 days, at the end of which time they were harvested and subjected to measurement and analysis. The plant material was frozen prior to the physico-chemical and chemical measurements.

In previous experiments, seedling variation had been found to be great, the probable error varying from $\pm$ 15 to $\pm$ 45 per cent. of the means, de-

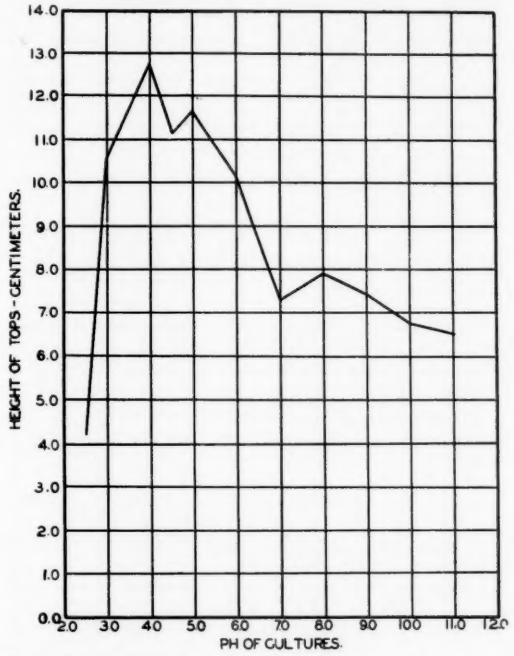


FIG. 1. Effect of reaction on height growth.

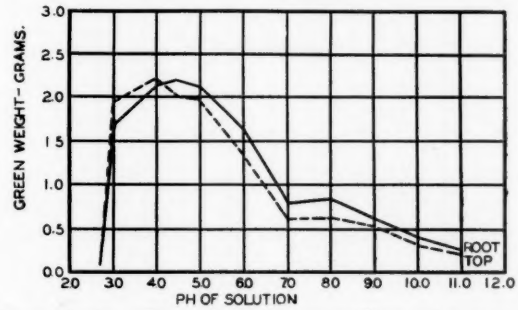


FIG. 2. Curves showing effects of reaction on seedlings of western yellow pine.

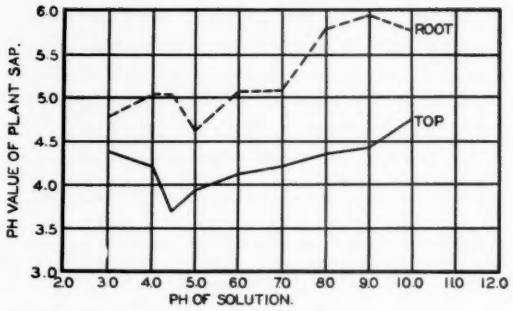


FIG. 3. Curves showing effects of reaction on the pH values of the plant sap.

pending on the measure. The measures for height growth had the smallest probable error; those for the green weights and dry weights showed the largest probable error.

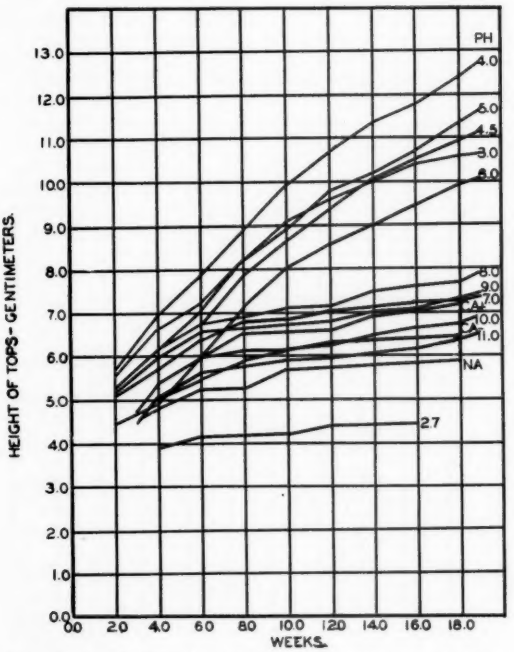


FIG. 4. March of height growth for seedlings; each curve indicates a definite pH reaction.

Results

In this experiment it was found that seedlings of western yellow pine would exist over an extensive range of reaction (figs. 1, 2, 3, 4). The growth limit on the acid side was at pH 2.7, but the growth limit was unde-

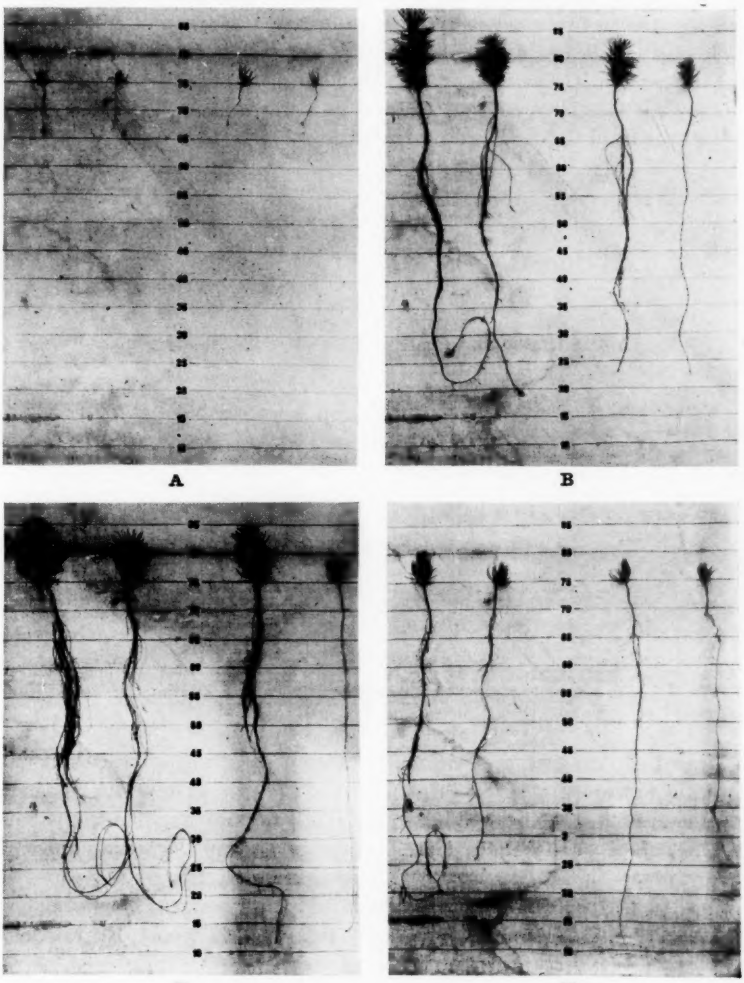
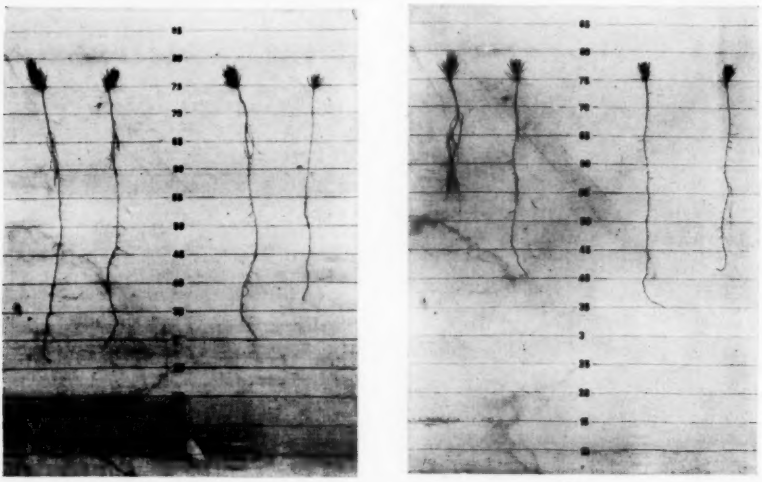


FIG. 5. Western yellow pine seedlings grown in culture ranging in pH from 2.7 to 7.0: *A*, pH 2.7; *B*, pH 4.0; *C*, pH 5.0; *D*, pH 7.0.

termined for the alkaline range. However, a few plants existed in cultures of pH 11.0 for the term of the experiment, and this may be taken as the limit of growth for the alkaline range. This gives a total range of reaction of from pH 2.7 to pH 11.0.

The general appearance of the plants growing in the acid solutions was striking when contrasted with those grown in the alkaline cultures (figs. 5, 6). The seedlings in the acid solutions were vigorous and well developed while those in the alkaline cultures were stunted and chlorotic.

Physical measurements of the seedlings were taken at the termination of the growth period and are presented in table III.



A B

FIG. 6. Western yellow pine seedlings grown in culture solutions of 9.0 and 10.0: *A*, pH 9.0; *B*, pH 10.0.

Height growth measurements were taken at intervals of seven days and present an interesting set of growth curves (fig. 4). Three distinct bands are formed; namely, the acid band, the alkaline band, and the single curve for the acid growth limit. These curves give ample evidence as to the efficacy of the acid cultures. The growth rate of the plants was little diminished at the termination of the experiment, whereas in the alkaline cultures the rate of growth was greatly retarded. The plants growing in the very acid culture (pH 2.7) had a negligible growth rate.

The length of the tap root was affected by the reaction of the culture. Those in the acid media appeared healthy and vigorous, whereas those in the alkaline solutions were brown and thickened, with a tendency to disin-

TABLE III

SUMMARY OF PHYSICAL AND CHEMICAL MEASUREMENTS OF SEEDLINGS

Measurements	pH 2.7*		pH 3.0		pH 4.0		pH 4.5		pH 5.0		pH 6.0
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
1. Number of plants	7	7	29	29	33	33	32	32	32	32	34
2. Height of tops (cm.)	4.400	—	10.6000	—	12.7600	—	11.0400	—	11.6200	—	10.1200
3. Length of roots (cm.)	—	6.870	—	50.8000	—	65.6000	—	69.4000	—	78.3000	—
4. No. of lateral roots	—	6	—	72.6000	—	85.0000	—	87.7000	—	92.1000	—
5. Diameter of stem (mm.)	0.940	—	2.1000	—	2.4300	—	2.2900	—	2.4100	—	2.2800
6. Green weight (gm.)	0.097	0.054	1.6900	1.9200	2.1500	2.2000	2.1600	2.0000	2.0900	1.9500	1.6100
7. Top-root ratio	1.790	—	0.8800	—	0.9800	—	1.0800	—	1.0700	—	1.2000
8. Yield of sap (ml.)	—	—	25.0000	37.8000	52.0000	34.5000	41.0000	30.5000	38.5000	27.5000	28.5000
9. Weight of 1 ml. of sap	—	—	1.0130	1.0080	1.0230	1.0080	1.0300	1.0040	1.0350	1.0060	1.0350
10. Index of refraction	1.356	—	1.3440	1.3380	1.3450	1.3390	1.3420	1.3380	1.3460	1.3390	1.3490
11. Percentage of solids	15.100	—	7.5000	3.2000	8.3000	4.4000	6.4000	3.3000	8.7000	4.1000	10.5000
12. Weight of solids (gm.)	—	—	0.0760	0.0320	0.0850	0.0440	0.0660	0.0330	0.0900	0.0410	0.1080
13. Freezing-point depression	—	—	0.9340	0.4440	0.9960	0.5770	0.7770	0.4520	0.9570	0.6030	1.0500
14. Osmotic pressure (atmos.)	—	—	11.2500	5.3400	11.9900	6.9500	15.4300	10.2000	11.5200	7.2600	12.6400
15. Mean molecular weights	—	—	163.1000	139.4000	181.5000	152.1000	165.8000	141.6000	187.2000	133.4000	206.7000
16. Specific conductivity (k)	—	—	0.0007	0.0005	0.0008	0.0007	0.0006	0.0006	0.0007	0.0007	0.0012
17. Saponification number	—	—	14.2200	8.8900	15.1500	9.7200	15.4300	10.2000	17.5000	8.5600	19.5200
18. Acid number	—	—	6.0200	4.8400	7.0100	6.0500	5.2300	3.7800	7.1900	4.5700	8.7500
19. Ether number	—	—	7.2000	4.0500	8.1400	3.6700	10.2000	6.4200	10.3100	4.0100	10.7700
20. Iodine number	—	—	1.3300	0.6600	2.1200	0.5500	1.3000	0.5100	1.5400	0.9000	1.7300
21. pH value of sap	—	—	4.3900	4.7800	4.2200	5.0300	3.7000	5.0100	3.9200	4.5500	4.1200
22. N/10 NaOH (ml.) to neutralize 5 ml. sap†	—	—	3.3400	2.0600	3.7000	2.2000	3.4500	1.8100	4.3700	2.2600	3.7500
23. N acid (ml.) neutralized per plant	—	—	—	0.7100	—	0.6500	—	0.5700	—	0.7300	—
24. N alkali (ml.) neutralized per plant	—	1.410	—	—	—	—	—	—	—	—	—

* Insufficient material for examination.

† Point of neutralization taken as pH 8.3.

TABLE III (*Continued*)

Measurements	pH 6.0	pH 7.0		pH 8.0		pH 9.0		pH 10.0		pH.11.0	
	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
1. Number of plants	34	18	18	19	19	21	21	8	8	2	2
2. Height of tops (cm.)	——	7.2300	——	7.8900	——	7.4000	——	6.8500	——	6.500	——
3. Length of roots (cm.)	89.700	——	57.8000	——	56.8000	——	46.8000	——	29.6000	——	31.700
4. No. of lateral roots	89.700	——	57.2000	——	55.2000	——	51.0000	——	31.9000	——	36.000
5. Diameter of stem (mm.)	——	1.7800	——	1.9300	——	1.6600	——	1.3600	——	1.500	——
6. Green weight (gm.)	1.340	0.7500	0.6000	0.8600	0.6200	0.5900	0.5100	0.3900	0.2900	0.240	0.200
7. Top-root ratio	——	1.2500	——	1.3900	——	1.1600	——	1.3200	——	1.200	——
8. Yield of sap (ml.)	21.500	9.0000	7.5000	10.0000	10.0000	6.0000	7.0000	5.0000	4.0000	%	%
9. Weight of 1 ml. of sap	1.015	1.0140	0.9960	1.0290	0.9780	1.0140	0.9630	1.0130	0.9720	%	%
10. Index of refraction	1.339	1.3460	1.3390	1.3490	1.3370	1.3450	1.3370	1.3510	1.3390	1.351	1.338
11. Percentage of solids	4.500	9.0000	4.3000	10.8000	2.8000	8.2000	2.8000	11.9000	3.7000	11.800	3.500
12. Weight of solids (gm.)	0.056	0.0910	0.0430	0.1110	0.0270	0.0840	0.0270	0.1210	0.0360	%	%
13. Freezing-point depression	0.678	0.8050	0.4650	1.1080	0.3240	0.8530	0.3770	0.6980	0.3540	%	%
14. Osmotic pressure (atmos.)	8.170	9.6900	5.6000	13.3400	3.9100	12.2600	4.5400	8.1400	4.2700	%	%
15. Mean molecular weights	128.400	231.1000	187.3000	204.0000	166.7000	199.7000	143.9000	226.6000	198.0000	%	%
16. Specific conductivity (k)	——	0.0009	0.0008	0.0013	0.0008	0.0013	.0008	0.0006	.0003	%	%
17. Saponification number	8.580	15.1000	6.7000	16.3000	4.3000	14.0000	5.2000	5.6000	2.2000	%	%
18. Acid number	5.510	8.2000	4.5000	8.4000	3.1000	6.6000	2.3000	4.6000	1.9000	%	%
19. Ether number	3.070	6.9000	2.2000	7.9000	0.9000	7.4000	2.9000	1.0000	0.3000	%	%
20. Iodine number	1.170	2.1000	0.7500	1.6500	0.3300	1.8200	0.3400	——	0.2000	%	%
21. pH value of sap	5.050	4.2100	5.1200	4.3600	5.7700	4.4100	5.9700	4.7400	5.7300	%	%
22. N/10 NaOH (ml.) to neutralize 5 ml. sap†	2.000	3.9000	1.0000	4.4000	1.7000	2.8000	0.8000	1.9500	0.8500	%	%
23. N acid (ml.) neutralized per plant	0.290	——	——	——	——	——	——	——	——	——	——
24. N alkali (ml.) neutralized per plant	——	——	0.1000	——	3.4200	——	7.9900	——	18.6900	——	21.580

* Insufficient material for examination.
† Point of neutralization taken as pH 8.3.
% Sap mixed with that from the plants grown at pH 10.0.

tegrate. The roots of the plants growing in the cultures of pH 2.7 were thickened and brittle. The number of lateral roots followed the same trend as the length of tap roots, and therefore it is reasonable to assume that they were dependent on this factor.

The dry weights of plants have been considered as criteria for metabolism, but in this case it was not possible to obtain the dry weights. The green weights followed the trend of the height of tops, exhibiting the usual points of weakness. It was evident that the reaction of the culture medium influenced growth and development of the seedlings.

Immediately after harvesting, the plant material was frozen and, for 48 hours, held at a temperature of $-20°$ C. The frozen tissue was quickly thawed in hot water and the sap expressed in a small hand-press. The expressed sap, refrozen, was preserved for future studies.

The pH value of the mixed sap was determined by the hydrogen electrode (**8**), at the same time that the titration curves were made (fig. 7).

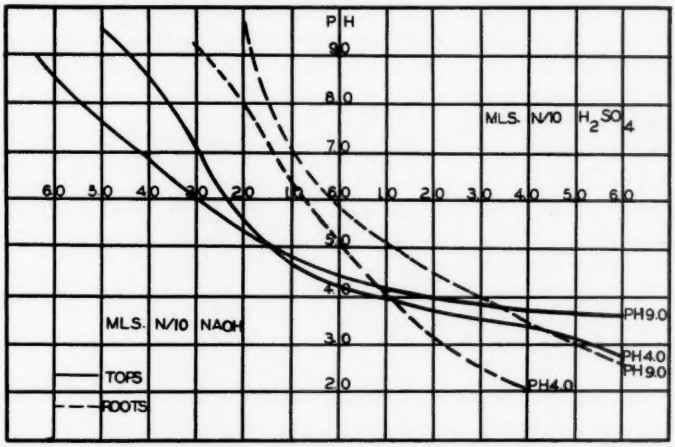


FIG. 7. Titration curves for the plant sap from the tops and roots of plants grown in the cultures at pH 4.0 and 9.0.

The pH values for the sap from the tops tended to vary from the acid to the alkaline side; however, the values decreased at pH 4.0, 4.5, and 5.0. The values for the sap from the roots followed the same trend as did those for the tops. In all cases the sap was found to be distinctly acid.

The titration curves for the sap, both of the tops and the roots, form definite and strong buffer curves. The sap from the roots had distinctly less buffer action than the sap from the tops. These results showed that the sap of the roots was more dilute or contained less material that was capable of acting as a buffer.

The freezing-point depressions were taken with a Haidenhain thermometer, standardized with freshly boiled distilled water. The resulting data showed that the sap was affected directly or indirectly by the reaction of the cultures.

The saponification number, acid number, and iodine number, all of which were found by using standard methods, varied and usually showed differences in the general trend at pH 4.5 and 7.0. The constituents of the sap possibly were altered as to quantity and quality. This alteration in the plant sap presumably affected the endurance of the plants toward the effects of reaction. The physiology of this point will be discussed in another section.

Other measurements were made, the results of which are given in table III.

A change in the pH values of the cultures was noted, with the result that the plants were found to influence the specific reaction. Plants are known to absorb ions (**1, 2, 7, 19, 21, 22, 23, 24, 29, 31, 36, 37**) from solution and thereby change its reaction. Also, the plant may excrete ions (**19, 25**) and in that way alter the pH value. Upon examination of the solutions for a decrease in the ions, it was found that absorption was negligible and could not cause any great change in reaction (table IV). The excretion of carbon dioxide was next studied in some detail, but the results were again negligible. No further examinations were made, but it was assumed that some organic substances were excreted by the seedlings, causing changes in the reaction. The magnitude of the change of reaction depends upon the buffer action of the solution. The potassium acid phosphate was the buffer agent in the solution, but this was precipitated in the alkaline solutions as insoluble calcium phosphate.

TABLE IV

ABSORPTION OF IONS BY THE SEEDLINGS

Ion	Milli-equivalents*			
	First set	Second set	Third set	Mean
Ca	0.180	0.095	0.075	0.117
PO_4	0.270	0.111	0.084	0.155
NO_3	0.166	0.055	0.056	0.092

* One hundred plants for 10 hours.

There was little doubt that the plants were instrumental in changing the reaction of the cultures in some manner, such as through the absorption or excretion of ions or molecules.

Certain investigators (**5, 26**) have stated that western yellow pine fails to grow when large quantities of lime are present in the soil. To determine the accuracy of this statement, one set of plants was grown in a culture containing no calcium; another in a culture containing an average of 1000 p.p.m. of calcium; and a third in a culture containing an adequate amount of calcium, with the alkalinity produced by the addition of sodium hydroxide. The solutions were kept at pH 9.0. The results were not greatly different in all cases, but the plants growing in the solutions containing an excess of calcium were superior to the other two. The sodium-treated plants were inferior to the others. The plants, according to this evidence, withstood large quantities of calcium; they also could exist in a solution deficient in calcium.

The leaves of the seedlings in the alkaline solutions were yellowish in color, and it was assumed that the plants could not assimilate and translocate sufficient iron (**37**). The metabolism of the plants seems to be disturbed in the alkaline media, thus producing a poor color in the leaves. Also, iron has been found to become insoluble after having been in solution for a reasonable time.

Discussion

Several investigators (**2, 28, 30, 33, 35, 41**) have casually studied the effects of soil reaction on forest trees, although little work has been carried on in detail. The opinion of European investigators is that the reaction of the soil is a potent factor in forest planting, natural regeneration, and forest production. The conifers were observed to prefer an acid reaction for their best growth, and soils under coniferous forests were found to be on the acid side of neutrality. Conifers did not do well on the alkaline soils, but certain tolerant species withstood an alkaline condition and competed with more resistant plants.

The concentration of the soil solution (**1, 2, 18, 20, 21, 22, 24, 39**) is an important factor in plant growth. Also there must be considered the concentration of each ion, since HOAGLAND (**19, 21**) and others have found that there is a minimum quantity which limits maximum growth. Small variations in the concentration do not appreciably affect the growth of the plants. The concentration of the soil solution also influences the concentration of the sap (**23**); it affects certain ions (**36**), and in turn alters the buffer system (**24, 29**).

The hydrogen-ion concentration, having been studied in reference to agricultural plants (**2, 40**), has been found to influence the rate of growth. A greater number of plants prefer a slightly acid medium for their best growth. The seedlings of western yellow pine prefer an even greater degree of acidity than do most cultivated plants. The absorption of nitrates

(**20**) has been found to be greater in the acid solutions, and would, therefore, increase the rate of growth of the seedlings.

Consequently, the behavior of western yellow pine seedlings in preferring acid soils is not different from that of other plants, the only difference being that they prefer a more acid medium. As found by WHERRY (**41**), most conifers prefer an acid soil rather than a neutral or alkaline one.

The pH value of the various plant tissues is characteristic (**3, 12, 13, 32**); the cell sap is usually acid and the protoplasm alkaline. The mixed sap for the western yellow pine seedlings was decidedly acid. However, no examination was made of the various tissues. The mixed saps were well buffered, as shown by the titration curves, although slight differences are to be noted. The buffer system of a plant may be affected by the external media, according to THERON (**37**) and others (**14, 29**), and this system is affected by the acid and alkaline reserves in the plant. Some investigators have concluded that certain specific substances are responsible for the buffer system. In the expressed sap of the plants used in this experiment, large quantities of catechol tannins were found, as well as other resinous materials.

The seedlings growing in the alkaline cultures were chlorotic and in some cases the yellow color was pronounced. Chlorosis has been attributed to many sources (**11**), but it was evident in this experiment that a lack of iron in the leaves was responsible. The plants either did not assimilate the element properly or failed to translocate it after assimilation. The kind of iron compound (**9, 11, 38**) and the type of iron have great influence on the ability of the iron to remain in solution. Even with the best of care it was not possible to relieve the situation. This appearance of the seedlings can be used as an indicator of adverse alkaline soils.

As has previously been stated, the plants were capable of changing the pH value of the culture medium, although the change was usually slight, except in the alkaline cultures. By what process the seedlings changed the reaction of the solutions remains unknown. Their ability to bring about this change does not mean that the plants would effect any great change in so well buffered a medium as the soil.

The constituents of the sap of the plants determine to some extent their ability to withstand environmental factors. Plants with a sap that is highly buffered will usually withstand a wide range of conditions. There are in evidence points which investigators (**2, 16**) have designated as isoelectric. In this case these points are at pH 4.5 and 7.0, and they were noticeably inferior to those in the cultures of lower and higher pH values. Plants subject to these conditions would therefore be inferior and less likely to withstand competition.

The hydrogen ion (**7, 10, 27**) has been considered more toxic than the hydroxyl ion, but it has been shown in this experiment that the reverse is true. Since such is the case in this study, the best forests should be in the regions of slightly acid soils, and the seedlings produced in alkaline materials would be inferior in form and color.

HESSELMAN (**15**) concludes that soil acidity is closely related to the form of available nitrogen. In this case then the plants should assimilate nitrogen in larger quantities in the acid solutions; and since the quantity of nitrogen used by western yellow pine seedlings is small, there should be little danger of competition.

Summary

1. Western yellow pine seedlings prefer an acid medium for their best growth.

2. Under field conditions other factors may have more influence on growth than the soil reaction.

3. The osmotic pressure of the sap indicates that soil reaction affects the seedlings as well as do certain other factors.

4. The buffer system of the seedlings is dependent on the organic materials found in the sap as well as on the inorganic substances.

5. The ability of the seedlings to change reaction is not to be construed as an effective measure in reducing soil alkalinity. Débris from the forest cover may be more effective because of the relatively high resin content.

6. Calcium is not deleterious to the seedlings. The fact that the plant does not assimilate the ion in alkaline soils may be of more importance.

7. The seedlings withstand a diversity of conditions, especially in regard to soil acidity and alkalinity.

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ABSORPTION OF OXYGEN BY ROOTS WHEN THE SHOOT IS IN DARKNESS OR IN LIGHT

W. A. CANNON

The writer has observed a variation in the oxygen content of the substratum of roots which appears to be related to certain of the environmental conditions of the shoot, especially to light and darkness, to temperature and to the relative humidity, or to the rate of evaporation. It had been observed previously, also, that there might be a direct relation, at least in time, between root growth and the lighting conditions of the shoot,[1] although in the preliminary experiments here referred to no relation was found between the rate of root growth and the moisture condition of the air. The present paper gives an account of experiments on the possible relation of the shoot to light and darkness and the variation of the oxygen content of the root environment.

As to the results, it can be premised that they suggest the probability that roots may obtain the oxygen used by them from at least two sources, namely, (1) from their immediate environment and (or) (2) from the shoot. In the latter event it is probably to be regarded as a product of photosynthesis. Thus, in the root, and also in the plant as a whole, autoaeration may in part obtain. Oxygen presumably derived from photosynthesis has, for convenience, been referred to as "internal" oxygen[2] and may be an important feature in the aeration of the tissues of all green plants.

Experiments

The experiments which are summarized in this paper were carried out[3] in March–September, 1931 and 1932, at Stanford University. Although in the course of the work several species were used, the number was finally reduced to two, namely, a willow (*Salix laevigata* Bebb) and the sunflower (*Helianthus annuus* L.). The former occurs along water courses on the campus of the University and the latter was obtained in a local market; the variety is not known.

Water cultures were used. The plants were grown in Knop's solution but were transferred for tests to distilled water. This was done because

[1] CANNON, W. A. Year Book, Carnegie Inst. Washington, no. 24, 1925; no. 30, 1930; Physiological features of roots, Pub. no. 368, Carnegie Inst. Washington; and On the variation of the oxygen content of culture solutions. Science **75**: 108–109. 1932.

[2] BEARDSLEY, G. F., and CANNON, W. A. Note on the effects of a mud-flow at Mt. Shasta on the vegetation. Ecology **11**: 326–336. 1930.

[3] The study was carried on at Stanford University with assistance of grants from the American Association for the Advancement of Science, in 1931, and the National Research Council, 1932.

in the Winkler method, which was used, nitrites interfere with determination of oxygen; and when Knop's solution stands for some time, nitrites appear in sufficient amount to invalidate the determinations.

The plants were kept in one-quart fruit jars which were made light-tight and which were well sealed. Each jar carried a thermometer, inlet and outlet tubes, and one or more plants. The tubes were closed with a short piece of rubber tubing and a pinchcock or clamp. The most satisfactory inlet tube was a small calcium chloride drying tube which was provided with a rubber stopper with one hole carrying a capillary tube. This arrangement accomplished two ends; it permitted the detection of defective sealing, and provided water to replace that taken up by the plant. When it was desired to have a solution with no oxygen (or very little) at the beginning of any test, this was accomplished by utilizing the solution in which a plant, such as a willow, had been growing in darkness for a period of 12 hours or more. By appropriate arrangement of the cultures it was possible to withdraw the water and run it into experimental jars without appreciable admixture of oxygen from atmospheric air.

In general the method of conducting the experiments was to keep the plants under observation in darkness or in light for equal periods, usually from 1.5 to 3 hours. During the light period, preferably at midday, the plants were directly exposed to sunlight; or they were shaded lightly by wire-mesh screens; or they were covered with a bell glass which was placed either in direct sunlight or under the screens. When bell glasses were used, cooling was effected by running water. The results from artificial lighting were entirely negative, and no report is given of such experiments.

The Thompson-Miller apparatus for the micro-determination of dissolved oxygen by Winkler's method was used.[4] The only change in the apparatus found advisable was the introduction of a glass bead into the sample chamber to assist in the mixing of the ingredients.

The sample chamber had a capacity of about 5 cc. but it usually varied very considerably so that calibration was necessary.

In tables I and II are given the most important data derived from the study of willow and sunflower. The willows were cuttings which had been rooted and were kept in a nutrient solution except during actual tests. The sunflower seedlings were treated similarly.

[4] Ind. & Eng. Chem. 1928. See also SNOKE, A. W. The determination of dissolved oxygen with the micro-Winkler apparatus of Thompson and Miller. Ecology **10**: 163–164. 1929. A further account is by ZIMMERMAN, Rept. and Proc. IXth International Congress, London, 7th to 15th August, 1930. For the general method see SUTTON'S Volumetric analysis, p. 480. 1924; Standard methods of the examination of water and sewage, 2d ed., 1912, American Public Health Assoc.; and the critique by ALSTERBERG, G. Die Winklersche Bestimmungsmethode für in Wasser gelösten, elementaren Sauerstoff sovie ihre Anwendung bei Anwesenheit oxydierbarer Substanzen. Biochem. Zeitschr. **170**: 30–75. 1926.

TABLE I

AMOUNT OF OXYGEN REMOVED BY ROOTS FROM DISTILLED WATER WHEN THE SHOOT HAD BEEN IN DARKNESS OR IN SUNLIGHT FOR GIVEN PERIODS OF TIME

I. *Helianthus annuus**

Exp. no.	Temperature			Time period	Oxygen consumption per liter	
	Control	Culture	Air		Darkness	Sunlight
	°C.	°C.	°C.		mg.	mg.
1	21.5	24.5	25.5	8 to 10 A. M.	.....	2.94
	21.5	23.0	22.5	11 A. M. to 2 P. M.	1.91	.....
	24.0	24.5	24.0	2 P. M. to 5 P. M.	.....	2.40
2	21.5	22.0	22.5	8 to 11 A. M.	4.40	.....
	21.5	25.5	30.0	11 A. M. to 2 P. M.	.....	0.00
	24.0	23.0	23.2	2 to 5 P. M.	2.56	.....
3	19.5	24.5	25.0	8 to 11 A. M.	.....	2.80
	21.0	23.0	23.0	11 A. M. to 2 P. M.	1.68	.....
	25.0	25.0	23.5	2 to 5 P. M.	.....	0.40
4	19.5	21.5	22.0	8 to 11 A. M.	2.06	.....
	21.0	25.0	31.0	11 A. M. to 2 P. M.	.....	2.36
	25.0	23.5	25.0	2 to 5 P. M.	1.41	.....
5	19.0	22.0	22.0	8 to 11 A. M.	0.84	.....
	23.0	24.0	23.0	11 A. M. to 2 P. M.	.....	2.23
	26.0	24.0	23.0	2 to 5 P. M.	2.55	.....
6	19.0	22.5	22.0	8 to 11 A. M.	.....	2.75
	23.0	23.0	23.0	11 A. M. to 2 P. M.	2.20	.....
	26.0	23.5	22.5	2 to 5 P. M.	.....	3.02
7	18.0	19.5	27.0	8 to 10 A. M.	0.17	.....
	18.5	21.0	24.0	10 A. M. to 12 M.	.....	0.00
	21.5	21.0	24.0	12 M. to 2 P. M.	0.40	.....
	23.0	21.0	24.0	2 to 4 P. M.	.....	1.29
	23.5	21.5	18.5	4 to 6 P. M.	0.49	.....
8	18.0	21.5	20.0	8 to 10 A. M.	.....	0.39
	18.5	20.5	20.0	10 A. M. to 12 M.	0.88	.....
	21.5	21.5	24.0	12 M. to 2 P. M.	.....	0.00
	23.0	22.0	21.0	2 to 4 P. M.	2.64	.....
	23.5	20.5	24.0	4 to 6 P. M.	.....	3.14
9	18.5	21.0	22.5	8 to 11 A. M.	3.47	.....
	20.0	26.0	24.0	11 A. M. to 1 P. M.	.....	1.91
10	18.5	21.0	22.5	8 to 11 A. M.	2.71	.....
	20.0	25.0	24.0	11 A. M. to 2 P. M.	.....	1.15
11	16.0	20.5	21.5	8 to 11 A. M.	3.58	.....
	18.0	24.5	24.0	11 A. M. to 2 P. M.	.....	2.35
12	16.0	20.5	21.5	8 to 11 A. M.	2.37	.....
	18.0	24.5	24.0	11 A. M. to 2 P. M.	.....	1.36

* The shoot was kept under a bell glass when exposed to the light and also either under a bell glass or under a tight cover when in the dark. In any case the atmosphere about the shoot was saturated, or nearly so.

TABLE I—(*Continued*)

Exp. no.	Temperature			Time period	Oxygen consumption per liter	
	Control	Culture	Air		Darkness	Sunlight
	°C.	°C.	°C.		mg.	mg.
13	15.0	18.5	21.5	8 to 11 A. M.	4.39	.....
	18.0	24.0	24.0	11 A. M. to 2 P. M.	.....	1.86
14	15.0	18.5	21.5	8 to 11 A. M.	1.87	.....
	18.0	24.0	24.0	11 A. M. to 2 P. M.	.....	0.00
15	16.5	19.0	19.0	8 to 11 A. M.	2.53	.....
	17.0	23.0	20.5	11 A. M. to 2 P. M.	.....	1.19
16	16.5	19.0	19.0	8 to 11 A. M.	1.92	.....
	17.0	23.0	20.5	11 A. M. to 2 P. M.	.....	1.64
17	11.5	17.0	20.0	8 to 11 A. M.	4.18	.....
	13.5	24.0	21.0	11 A. M. to 2 P. M.	.....	1.24
18	11.5	17.0	20.0	8 to 11 A. M.	2.27	.....
	13.5	19.5	21.0	11 A. M. to 2 P. M.	.....	1.37
19	14.0	17.5	20.0	8 to 11 A. M.	2.83	.....
	15.0	22.0	15.0	11 A. M. to 2 P. M.	.....	2.53
20	14.0	17.5	20.0	8 to 11 A. M.	1.62	.....
	15.0	22.5	22.0	11 A. M. to 2 P. M.	.....	0.72
21	17.0	18.0	19.5	8 to 11 A. M.	2.93	.....
	17.0	23.0	24.0	11 A. M. to 2 P. M.	.....	4.71
22	18.0	19.5	25.0	8 to 11 A. M.	3.03	.....
	18.0	23.0	24.0	11 A. M. to 2 P. M.	.....	2.90
23	18.0	19.5	25.0	8 to 11 A. M.	2.49	.....
	18.0	24.0	24.5	11 A. M. to 2 P. M.	.....	2.70
24	18.0	19.5	25.0	8 to 11 A. M.	2.76	.....
	18.0	24.0	24.5	11 A. M. to 2 P. M.	.....	1.89
25	23.8	23.5	26.5	8 to 10: 30 A. M.	5.60	.....
	20.5	20.0	26.5	11: 30 A. M. to 2 P. M.	.....	6.27
26	18.8	23.0	23.0	8 to 9: 30 A. M.	4.53	.....
	21.7	23.0	23.9	9: 30 to 11 A. M.	.....	4.02
	23.5	23.5	25.0	12: 55 to 2: 25 P. M	.....	4.78

Discussion

This study is concerned with the rate at which roots absorb dissolved oxygen when the shoot is either in darkness or in light, especially in direct sunlight. Twenty-six experiments were carried out on the sunflower and twenty-seven on rooted willow cuttings. Of the 53 experiments summarized in tables I and II, 60 per cent. showed a slower rate of oxygen consumption by the roots when the shoot was in sunlight. In the sunflower the rate was

TABLE II

OXYGEN REMOVED BY ROOTS FROM DISTILLED WATER WHEN THE SHOOT HAD BEEN IN DARKNESS OR IN SUNLIGHT FOR GIVEN PERIODS OF TIME

II. *Salix laevigata*

EXP. NO.	TEMPERATURE			TIME PERIOD	OXYGEN CONSUMPTION PER LITER	
	CONTROL	SOLUTION	AIR		DARKNESS	LIGHT
	°C.	°C.	°C.		mg.	mg.
27	23.4	23.0	.....	8 to 11 A. M.	4.12	.....
	22.2	22.0	.....	11 A. M. to 2 P. M.	.....	3.75*
28	24.0	24.0	.....	8 to 11 A. M.	4.14	.....
	24.8	24.5	22.2	11 A. M. to 2 P. M.	.....	5.32*
29	24.0	24.0	.....	8 to 11 A. M.	3.26	.....
	24.0	24.0	.....	11 A. M. to 2 P. M.	.....	1.71*
30	24.6	22.5	.....	8 to 11 A. M.	3.18	.....
	21.5	21.0	.....	11 A. M. to 2 P. M.	.....	2.66*
31	22.0	22.0	.....	8 to 11 A. M.	5.90	.....
	23.0	22.0	.....	11 A. M. to 2 P. M.	.....	4.70*
32	23.5	22.5	.....	8 to 11 A. M.	4.40	.....
	22.7	23.0	.....	11 A. M. to 2 P. M.	.....	5.60*
33	23.0	22.5	.....	8 to 11 A. M.	3.82	.....
	26.0	24.0	.....	11 A. M. to 2 P. M.	.....	4.42*
34	22.8	23.0	.....	8 to 11 A. M.	1.19	.....
	23.7	24.0	.....	11 A. M. to 2 P. M.	.....	1.12*
35	22.5	22.0	24.5	8 to 11 A. M.	6.93	.....
	22.0	22.5	23.9	11 A. M. to 2 P. M.	.....	7.38*
36	23.0	22.5	22.5	8 to 9: 30 A. M.	6.04	.....
	22.5	23.0	.....	9: 30 to 11 A. M.	.....	5.20*
	23.3	23.5	26.7	12: 30 to 2 P. M.	.....	5.44*
37	22.7	22.0	22.0	8 to 9: 30 A. M.	5.32	.....
	22.8	23.0	.....	9: 30 to 11 A. M.	.....	5.44*
	22.0	22.0	25.0	1 to 2: 30 P. M.	.....	4.48*
38	23.4	23.4	23.4	8 to 10 A. M.	5.62	.....
	23.0	24.4	.....	10 A. M. to 12 M.	.....	5.12*
	24.2	22.5	.....	12 M. to 2 P. M.	.....	5.58*
	23.3	22.5	.....	2 to 4 P. M.	.....	5.96*
39	21.5	21.0	21.0	8 to 9: 30 A. M.	4.42	.....
	20.0	21.0	22.2	9: 30 to 11 A. M.	.....	3.43*
	21.6	21.5	23.3	1 to 2: 30 P. M.	.....	4.00*
40	20.8	20.0	23.0	9 to 10: 30 A. M.	3.24	.....
	21.0	21.0	17.2	10: 30 A. M. to 12 M.	.....	5.30*
41	21.5	21.5	21.5	8 to 9: 30 A. M.	4.25	.....
	20.5	21.5	23.9	9: 30 to 11 A. M.	.....	5.22[b]
	22.7	26.0	24.4	12: 30 to 2 P. M.	.....	5.52

TABLE II—(*Continued*)

Exp. no.	Temperature			Time period	Oxygen consumption per liter	
	Control	Solution	Air		Darkness	Light
	°C.	°C.	°C.		mg.	mg.
42	22.2	22.5	.....	8 to 9:30 A. M.	3.58	.....
	22.8	21.5	23.3	9:30 to 11 A. M.	.....	3.29[b]
43	22.5	22.0	.....	8 to 11 A. M.	5.82	.....
	21.5	22.0	23.3	11 A. M. to 2 P. M.	.....	5.25[b]
44	22.3	22.0	.....	8 to 11 A. M.	5.89	.....
	22.0	22.0	24.4	11 A. M. to 2 P. M.	.....	5.11[b]
45	20.5	20.5	20.5	8 to 11 A. M.	5.30	.....
	18.1	20.5	22.2	11 A. M. to 2 P. M.	.....	5.18[b]
46	20.3	20.0	23.0	8 to 11 A. M.	5.92	.....
	19.1	21.5	25.0	11 A. M. to 2 P. M.	.....	6.61
47	20.5	20.5	22.5	8 to 11 A. M.	6.71	.....
	19.1	20.0	23.3	11 A. M. to 2 P. M.	.....	6.24[b]
48	20.3	20.0	23.0	8 to 11 A. M.	5.94	.....
	22.3	21.0	20.6	11 A. M. to 2 P. M.	.....	5.79*
49	20.5	20.0	22.7	8 to 11 A. M.	6.43	.....
	22.3	21.0	28.9	11 A. M. to 2 P. M.	.....	6.96*
50	20.8	20.2	23.0	8 to 11 A. M.	5.90	.....
	22.9	24.0	23.3	11 A. M. to 2 P. M.	.....	6.50[b]
51	20.7	20.4	.....	8 to 11 A. M.	7.09	.....
	24.0	25.5	27.6	11 A. M. to 2 P. M.	.....	6.40[b]
52	24.2	24.0	24.5	8 to 11 A. M.	7.14	.....
	22.0	22.0	24.4	11 A. M. to 2 P. M.	.....	6.79[b]
53	23.3	23.0	22.0	8 to 9:30 A. M.	5.30	.....
	19.0	22.0	25.6	9:30 to 11 A. M.	.....	6.11[b]

* When the asterisk appears it signifies that the shoot was exposed directly to sunlight.

[b] When the letter *b* follows, it means that a bell glass was used over the shoot; otherwise the experiment was carried out in sunlight the same as in darkness.

reduced in 69 per cent. of the cases and in the willow in 59 per cent. A correlation therefore appears to obtain between the lighting conditions of the shoot and the rate of absorption of oxygen from the solution by the roots.

That there may be a shoot-root relation as regards oxygen absorption by roots is suggested by other observations. For example, when the possible effects of temperature and the evaporating power of the air were taken into account, no slowing of the rate was observed when the shoot was in

darkness, or in a well lighted room, or in the shade of a tree, or on days with weak sunlight, or in the early forenoon, or in the late afternoon.

It appears, therefore, that in addition to proper conditions of illumination at least two other factors must be favorable in order to bring about the retardation of the rate of oxygen absorption. Inasmuch as the consumption of oxygen by the roots is a feature of respiration, it would be expected that its rate would depend in part, at least, on temperature. An increase in temperature would operate in the direction of increasing the rate of respiration, and a temperature decrease would have the opposite effect. That is to say, in the present instance the former temperature condition would induce a relatively rapid rate of absorption of oxygen by the roots while the latter condition would have a retarding effect. And such effects were noted. In addition, the temperature must be specifically favorable for the most active rate of respiration, which probably means that the temperature optima for the two species here used would not be the same, that of the willow being somewhat lower. Such conditions were recognized as being of importance in connection with the study, and as accounting for the effects of temperature, whichever way operating. Yet it was found that when temperature was favorable, and when the humidity of the air also was favorable, there was usually the slowing effect above noted, provided the shoot was properly illuminated.

It will be well here to speak of the effects of rapid evaporation on the absorption of dissolved oxygen by roots. Data from one or two experiments will illustrate this. Thus in one experiment with willow, the shoot was exposed to direct sunlight from 9 A. M. to 3 P. M. From 11 A. M. to 1 P. M. a current of air from a small electric fan was caused to play on the shoot. During the period when the fan was in use the rate of oxygen absorption increased about 50 per cent. In another and similar experiment the increase was about 200 per cent. In general it was found that when the rate of evaporation was fairly high, as often is the case in summer in this vicinity, the rate of oxygen absorption by the roots was relatively rapid. For this reason when the experiments were carried on in sunlight, negative results were often obtained; that is, there was no decrease in the rate of the consumption of oxygen by the roots. In many experiments with the willow, however, especially those in early spring, there was found to be a decrease in the rate of consumption of oxygen with the shoot in sunlight, notwithstanding the moderate evaportion rates then obtaining. But in no case, when the shoot was wilted, was there a slowing of the rate of oxygen absorption. The relation between the dryness of the air and the high rate of absorption of oxygen by roots was so constant that it was usually found necessary to protect the shoot with a bell glass. It can be said, therefore, that whenever the dryness of the air, or the temperature of the air or of the

solution did not prevent, there was a distinct slowing of the rate of the removal of oxygen from the solution by the roots during the period in which the shoot was exposed to the sunlight.

In addition to the foregoing, the work of CERIGHELLI[5] might be cited as supporting the main thesis here advanced, although it is but fair to say that the interpretation of the results of that author are my own.

CERIGHELLI studied the respiration of the roots of different species of plants under different conditions. The following table, compiled from his results, summarizes the carbon dioxide-oxygen ratios, $\frac{CO_2}{O_2}$, expressed decimally, with the shoot in darkness and in light, as well as in dry and moist air.

PLANT	DARKNESS	LIGHT
Zea mays	0.68	1.11
Lupinus albus	0.60	0.80
Ricinus communis	0.88	1.07
" "	0.91	1.00
	Shoot in dry air	Shoot in moist air
Malva sylvestrum	0.19	0.62
" "	0.20	0.66
Phaseolus multiflorus	0.37	0.56

It appears, therefore, that CERIGHELLI found that there was relatively more oxygen consumed by the roots when the shoot was in the dark and also when it was in dry air, as opposed to the relatively small consumption when the shoot was in the light and also when in moist air. He accounts for the apparently, and perhaps actually, larger evolution of carbon dioxide, when the shoot is in light, on the assumption that "le gaz carbonique circulerait de la feuille a l' organe souterrain," as indicated by the larger ratio in light. But he gives no explanation of the analogous condition when the shoot is in moist air.

The interest centers in the variation in the carbon dioxide-oxygen ratios under the conditions of darkness and of light. Variation in intensity does not affect this ratio. As regards the difference in the ratios under the dark or light conditions, it is evident that two quite unlike processes may be dealt with. In the first case the only available oxygen to the roots, and probably also to the shoot, is the atmospheric oxygen either of the soil or dissolved in the solution containing the roots, or that of the atmosphere itself. But in the second case there is, in addition, the oxygen which may originate in the green tissues through photosynthesis. Should this oxygen,

[5] CERIGHELLI, R. Recherches physiologiques sur la respiration de la racine. Annales de la Faculté des Sciences de Marseille. 1921.

or a portion of it, remain within the plant and move away from the chlorophyll-bearing organs, it would play a part in the aeration of the plant, whether stem or root. In this event an apparent or an actual decrease in the consumption of oxygen by roots might be expected to take place. And, in fact, this would be indicated by an upset in the carbon dioxide-oxygen ratio by which the proportion of carbon dioxide given off exceeds that of oxygen taken in, as CERIGHELLI found. The ratio would thus be a large one owing not to a necessarily larger evolution of carbon dioxide but to a relatively small absorption of oxygen from the root substratum, for the reason that a portion of the oxygen used in respiration, during the periods of light, originated, as above suggested, within the plant itself. Thus to this extent the plant is autoaerated. In the present study a decrease in the rate of oxygen consumption under such conditions was determined, but in that of CERIGHELLI a larger ratio was found. Thus the two investigations apparently supplement each other and point to the same conclusion.

The finding of CERIGHELLI also that in dry air the ratio is smaller than when the shoot is in moist air harmonizes with the results of the present study, as will be concluded from data presented earlier. That is to say, it was found that the rate of absorption of oxygen by roots, in appropriate light, and with favorable temperature conditions was, however, greatly increased with increase in the rate of evaporation. Attention should be called here to the following facts. It is known that the rate of photosynthesis is more rapid in turgid than in flaccid leaves. Also it was found, when the water content of the leaves of *Bidens tripartita* was reduced to about 44 per cent., that the rate of photosynthesis was reduced from 53 to 78 per cent., and, in *Phlomis pungens* a reduction of 34 per cent. in the water content brought about a 13 per cent. reduction in the rate of photosynthesis.[6] From the present point of view the most interesting feature of these relations is the decrease in the rate of formation of oxygen which would accompany a reduced rate of photosynthesis, and thus a possible diminution in the amount of oxygen directly available to the plant, including the roots. The possible chain of events leading to this would include, among other features, (1) an excessive demand by the leaves on the roots for water leading to an inadequate supply; (2) a diminution in the water content of the parenchyma including the chlorenchyma; (3) the closing of the stomata; and (4) a diminution in or a cessation of the intake of carbon dioxide. There would be a decrease or a cessation of photosynthesis, and with it a diminution or complete stoppage of the formation of oxygen as a photosynthate. The closing of the stomata would also directly affect transpiration and possibly all molar movements within the

[6] MILLER, E. C. Plant Physiology. p. 467. 1931. See also SPOEHR, H. A. Photosynthesis. p. 153. 1926.

plant; and therefore the diffusion of oxygen from the chlorophyll-bearing organs to the stem and to the roots would cease, or occur at a slow rate. Thus there would be less oxygen available to the roots and there might be, as appears to occur, a correspondingly large demand on the substratum to make up the deficiency. This would not only bring about a relatively large loss of oxygen from the substratum (or, in this case, the solution), but it might, as CERIGHELLI showed, result in a relatively small carbon dioxide-oxygen ratio.

It thus appears probable from the results of the present study, as well as from those of CERIGHELLI, that a green plant may derive a portion of the oxygen used by it in oxidative processes from the shoot when the latter is in light of appropriate intensity, when the temperature is suitable, and when the evaporation conditions affecting the shoot are not intense. It is probable, therefore, that the root as well as the shoot is to a certain degree autoaerated. Oxygen of such derivation may for convenience be called "internal" oxygen, as opposed to atmospheric oxygen which enters the plant, including the root, without organic transformation.

The evidence given in earlier paragraphs that this internal oxygen may supplement atmospheric oxygen in the aeration of the roots, and of the stem, is, of course, inferential. There appears little experimentation bearing directly on the subject. Of these the most familiar is that with *Elodea* in which the cut end of the stem gives off oxygen when the shoot is in sunlight. But it was also shown in earlier studies by the writer that oxygen may pass from the shoot to the root under appropriate environmental conditions, especially with good light.[7] To secure more positive evidence a few experiments along the same lines were carried out in connection with this study. Pending further work, the leading results to this time may be briefly presented. Sunflower plants and cut leafy branches of apricot were tested with the root, or the cut end of the stem, in oxygen-free distilled water. The experiments were carried on in general quite as those already reported. It was found in several experiments that there was an increase in the oxygen content of the cultures coincident with the exposure of the shoot to sunlight.

Although the experiments just summarized are, as suggested, of an introductory nature, they appear to indicate (1) that the partial pressure of oxygen of the stem, and probably also of the root, may increase when the plant is in sunlight; and (2) that at the same time this oxygen may escape into the substratum surrounding the root. It was shown earlier in this paper also that the rate of the consumption of oxygen by the root,

[7] CANNON, W. A. Year Book, Carnegie Institution of Washington. no. 24. p. 292. 1925.

for parallel time periods, might be less when the shoot is in sunlight than when it is in darkness.

It has already been stated that positive results were not always had from the exposure of the shoot to sunlight. Thus not only must temperature and humidity be taken into account but certain features of light as well. Thus the length of exposure to light may be too short, or it may be too early in the morning or too late in the afternoon, or it may precede a period of darkness of equal length, or it may be too long. In the last case the period of most favorable rate of photosynthesis has been passed and in the next preceding case there may be photosynthetic oxygen in the tissues in sufficient quantity to influence the readings for the dark period. But in the instances first postulated the reasons for negative results are obvious. Although not investigated from the present point of view, it is not unlikely that different species may show unlike reactions to the light variations above mentioned.[8] However this may be, it appears that the processes associated with photosynthesis start promptly upon exposure of the shoot to light.[9] Where this feature makes itself evident in the stem, as indicated by the early appearance of oxygen in the cut branches of apricot, or by the early escape of oxygen from the root of the intact sunflower, it connotes a fairly rapid movement of oxygen from the chlorophyll-bearing tissue to relatively distant organs. This presents no difficulty, however, as movement of water and of solutes in the direction of, or against, the transpiration stream, and laterally as well, is known to occur,[10] although the details of the picture at least as regards oxygen translocation remain to be filled in.

Such considerations suggest the desirability of studies on the aeration of plant tissues. Oxygen so used appears to have a multiple origin. It may enter the plant as a gas directly from atmospheric air, or it may enter the roots dissolved in soil water, or it may arise as a product of photosynthesis. Thus oxygen from the substratum may conceivably aerate the root and perhaps the stem, and that from atmospheric air may aerate the stem and perhaps the root, and photosynthetic oxygen may perhaps aerate both stem and root. What the relative amount of oxygen taking its origin from the several sources, or in the different ways, may be, is quite problematical. It would surely vary with conditions and it may vary with

[8] GARNER, W. W., and ALLARD, H. A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920. Also ADAMS, J. Relation of flax to varying amounts of light. Bot Gaz. **70**: 153–156. 1920, and later studies.

[9] *Cf.* CLEMENTS, H. F. Hourly variations in carbohydrate content of leaves and petioles. Bot. Gaz. **89**: 241–272. 1930.

[10] *Cf.* PEIRCE, G. J. The physiology of plants. p. 133. 1926, and MILLER, E. C. Plant Physiology. p. 669. 1931, for statements of the current views on movement of materials in plants.

the species. Certainly different species may not have the same light requirements, or the same oxygen requirements, and the possibility is that the two may be more closely related than is now commonly supposed. And, finally, it may well be that such specific differences in relation to oxygen in aeration may be important links in the chain of factors and events by which many species are adjusted to their special environment.

STANFORD UNIVERSITY.

COMPARISON OF EXISTING METHODS FOR THE DETERMINATION OF AMMONIA NITROGEN AND THEIR ADAPTABILITY TO PLANT JUICE[1]

FRANK S. SCHLENKER

(WITH ONE FIGURE)

Introduction

In 1927 GILBERT and HARDIN (**4**), and later GILBERT and SMITH (**5**), published data showing that there is a correlation between soil fertility and the chemical composition of expressed plant juice. MCCOOL and WELDON (**7**) and PETTINGER (**8**) in more recent work have substantiated the findings of GILBERT *et al.* The juice work of the Rhode Island workers has recently been extended to include the estimation of a number of the nitrogenous fractions. This has necessitated a study of methods, since the present chemical procedures used in plant work have been developed for water or alcoholic extracts of plant tissue. Accordingly the method for the determination of ammonia was chosen for adaptation to expressed juices, for the estimation of this plant constituent is one of the most frequent determinations made in the study of plants at this station.

Analytical methods

The initial ammonia methods used were those of FOLIN and BELL (**2**), the procedure as recommended by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists (**1**), and that of VICKERY and PUCHER (**10**).

The first method involves the shaking of the sample with permutit which takes up the ammonium ion by means of a base-exchange reaction, and the subsequent liberation by sodium hydroxide; the second, aeration after making strongly alkaline with 52 per cent. potassium carbonate; and the third, an 8-minute distillation with heat after the addition of heavy magnesium oxide. In the final portion of the investigation, VAN SLYKE'S (**9**) aeration-distillation method, using a saturated calcium hydroxide solution for the neutralizing agent, was employed. With the exception of FOLIN and BELL'S technique which requires Nesslerization, the ammonia of the remaining procedures can be estimated either by the use of titration methods or Nessler's solution. The procedure of the VICKERY and PUCHER method was slightly modified. The use of permutit to remove ammonia from the distillate was discarded since no interfering substance, as nicotine

[1] Published by permission of the Director of the R. I. Agricultural Experiment Station as Contribution no. 425.

in the original procedure, distills with the ammonia from plant juices. In addition the distillation was carried on by the use of an electric hot plate. By using an electric plate the amount of heat can be regulated more easily than with a micro-burner.

MATERIALS.—The plants for this study were obtained from the plats of the agronomy farm and from the college greenhouse. The leaves were treated as recommended by FREAR (3), except that after being wrapped as tightly as possible in cheesecloth they were placed in the carbo-freezer and frozen by a direct stream of carbon dioxide rather than indirectly by dry-ice cakes. After complete thawing the juice was expressed in a hydraulic press.

Experimental

In order to obtain an idea of the composition of the material to be used, a few miscellaneous determinations were made; that is, the weight of 5 ml. of juice, the hydrogen-ion concentration, ash, the approximate ammonia content of mesophyll and midrib tissue were secured (table I). Mesophyll

TABLE I

DETERMINATION OF SOME JUICE CONSTITUENTS (BEETS)

	WEIGHT OF 5 ML. OF JUICE		ASH		AMMONIA NITROGEN		HYDROGEN-ION CONCENTRATION	
SAMPLE NO.	MESOPHYLL	MIDRIB	MESOPHYLL	MIDRIB	MESOPHYLL	MIDRIB	MESOPHYLL	MIDRIB
	gm.	*gm.*	*per cent.*	*per cent.*	*p.p.m.*	*p.p.m.*	*pH*	*pH*
1	5.0873	5.0980	.....	.....	24.4	24.0	6.50	5.71
2	5.0779	5.0799	2.05	1.89	14.1	13.7	6.42	5.79
3	5.1005	5.1003	1.88	1.87	20.1	19.9	6.33	5.71
Average	5.0885	5.0927	1.96	1.88	19.5	19.2	.....	.....

tissue, as discussed here, is that portion of the leaf remaining after removal of the midrib. This includes, besides mesophyll tissue proper, the lateral veins which are too small to remove.

It has been the usual practice to use a 5-ml. sample of juice and consider the weight as 5 grams, for the sampling error is considered to be greater than the error involved by assuming this weight. Actually in a juice containing 50 p.p.m. of ammonia nitrogen the error is approximately 1 p.p.m., or 2.0 per cent.

The procedure as recommended by the Committee on Methods (1), which for convenience will be termed the aeration method, has been used

for the ammonia determinations from the beginning of the fractionation of nitrogen in plant juice at this station.

KOBER and GRAVES (**6**) cite evidence which shows that the amount of ammonia carried over by a stream of air depends on the volume and rate of aeration. They found that 650 liters of air per hour recovered 99.7 per cent. of ammonia from an ammonium sulphate solution in 50 minutes, while 139 liters per hour recovered 97.4 per cent. in 90 minutes. To cover this portion of the procedure, a flow meter was calibrated by a Junkers gas meter and a curve for the relationship of time and volume constructed. The flow meter was then inserted in the system, the suction adjusted to 110–120 liters per hour, and the aeration continued for two hours.

The 2-hour aeration period is considerably more than that mentioned in the original method, but experimental results indicate that this length of time is necessary to carry over the ammonia present at this rate of aeration. In an effort to follow the course of the aeration reaction, time-concentration studies were made for the juices used in this study and for a solution of ammonium sulphate. The ammonia in an ammonium sulphate solution can be recovered quantitatively in a short period of time. The results for plant juices when plotted (fig. 1) show that at the end of two hours there is a distinct change in the type and rate of reaction, for at this point there is a break in the curve and the evolution of ammonia becomes less rapid thereafter. The curve for beet juice is the most striking, bringing out more clearly than the other two curves the important point to be derived from these experiments. The ammonia results obtained by aerating for two hours showed close agreement with those obtained by the use of permutit as discussed in the succeeding paragraph. As the change in the rate of evolution of ammonia appears at that point where the results of the two determinations coincide, it can be concluded that two hours' aeration is sufficient to measure the uncombined ammonia.

With the idea in mind that the continued appearance of ammonia with prolonged aeration might be due to the hydrolysis of some unstable nitrogenous compounds, permutit was employed to remove the ammonia but not the unstable nitrogenous products. FOLIN and BELL (**2**) demonstrated that ammonia could be removed quantitatively from urine by the use of permutit, even in the presence of 20 to 30 mg. of sodium chloride. WHITEHORN (**11**) found that substances other than ammonia were taken up by permutit. The most important of these were the amines and amino acids, lysine, histidine, and portions of tyrosine and arginine. As FOLIN pointed out that the extraction of ammonia from solution was limited by the concentration of sodium chloride, it was necessary to test the adsorbing power of permutit in a solution having an inorganic salt concentration approxi-

mating that of plant juice. It will be seen from table I that the ash content of plant juice is between 1.96 and 1.88 per cent., and this gives an average concentration of 19 mg. of inorganic salts per ml. of juice, a figure close to FOLIN's lower limit. The ash content does not measure the salt concentration exactly, because of the evolution of some of the more volatile inorganic constituents of the juice, so that these figures are probably low.

Two buffered salt solutions were made up to have hydrogen-ion and salt concentrations somewhat similar to the plant juices in question. In table II are recorded the results for recovery of ammonia by permutit adsorption.

TABLE II

RECOVERY OF AMMONIA NITROGEN BY PERMUTIT FROM SYNTHETIC SOLUTIONS CONTAINING 50 P.P.M. OF AMMONIA NITROGEN

	SOLUTION 1,* PH 6.20	SOLUTION 2, PH 5.49
	per cent.	*per cent.*
	97.50	99.60
	101.00	98.70
	99.50	100.00
	100.60	99.20
	99.50	100.00
	100.00	99.20
	98.50	100.00
	100.00	99.00
	99.50	99.50
Average	99.56	99.46

Solution A: sodium monohydrogen phosphate 11.857 gm. per liter.
Solution B: potassium dihydrogen phosphate, 9.078 gm. per liter.

Synthetic solution 1: pH 6.20, 50 p.p.m. ammonia nitrogen

Sodium monohydrogen phosphate (A) 150 ml.	1.770	gm.
Potassium dihydrogen phosphate (B) 350 ml.	3.185	"
Ammonium sulphate	0.097	"
Sodium chloride	5.000	"
Total inorganic salt concentration, 2.01%	10.052	" in 500 ml.

Synthetic solution 2: pH 5.49, 50 p.p.m. ammonia nitrogen

Sodium monohydrogen phosphate (A) 37.5 ml.	0.443	gm.
Potassium dihydrogen phosphate (B) 462.5 ml.	4.209	"
Ammonium sulphate	0.097	"
Sodium chloride	5.000	"
Total inorganic salt concentration, 1.95%	9.749	" in 500 ml.

In so far as the two mixtures can be considered representative of plant juice with respect to hydrogen-ion and salt concentration, there is no doubt that permutit can be used to remove ammonia from solution quantitatively.

FOLIN'S method as adapted to juice work consisted of shaking 5 ml. of juice with 2 gm. of washed permutit in a 50-ml. volumetric flask for 5 to 7 minutes, washing free from remaining juice by decantation, adding 20 ml. of water and 2 ml. of 10 per cent. sodium hydroxide, then 5 to 10 ml. Nessler's reagent and diluting to volume. In all cases where this method was used an "off" color was produced which gave varying intensities of yellow light. This interfering color seemed to vary with crops, being less noticeable for beets and more for celery. In some instances, with celery, after the release of ammonia by sodium hydroxide the supernatant liquid showed a decided yellow color which disappeared upon addition of acid. It is not soluble, however, in such organic solvents as caprylic alcohol, ether, or xylene. In spite of this interfering substance the method can be used with a fair degree of accuracy, provided the "off" color is not too great, after becoming accustomed to the point of color match. This particular point seems to be that place where there is a sudden change from light yellow to a darker shade of the same color in the unknown solution, when comparing it with the standard solution in a Duboscq colorimeter. Before this procedure can be adopted with a perfect degree of surety this difficulty must be overcome, but the color interference does not entirely dismiss the use of permutit. It may be useful as a rapid method if great accuracy is not required.

The permutit method may be made accurate by the addition of aeration to the procedure as follows: 2 gm. of washed permutit are placed in an aeration tube and 5 ml. of juice added. This is shaken for 5 to 7 minutes and then washed clear of remaining juice by decantation. The tubes are placed in the VAN SLYKE-CULLEN aspiration block, and 3 ml. of 10 per cent. sodium hydroxide and an anti-foam reagent added. The apparatus is then connected as usual and aspirated 90 minutes (table III). This is the minimum time that can be used with safety. The ammonia is caught by 3 ml. of 0.01 N acid and either transferred to a volumetric flask for Nesslerization and dilution, or titrated by standard base.

With any of the methods where distillation or aeration are involved anti-foam reagents must be used. Ninety-five per cent. alcohol in the VAN SLYKE and paraffin in the VICKERY and PUCHER method works very well. In the aeration method caprylic alcohol is efficient, but it distills into the acid tube readily, and upon the development of color by Nesslerization a cloudiness appears, provided too much alcohol is present. If, however, the color is not brought out until just before comparison, with quick manipulation the colors can be matched successfully. This latter procedure was

TABLE III

TIME NECESSARY TO LIBERATE AMMONIA FROM PERMUTIT BY ASPIRATION

MINUTES	JUICE AMMONIUM NITROGEN			PURE AMMONIUM SULPHATE SOLUTION 50 P.P.M. AMMONIUM NITROGEN	
	BEETS	BEETS	CELERY		
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
10	6.70	7.00	.....	.....	.....
20	10.00	12.10	.....	26.60	.....
30	12.50	14.70	.....	38.50	.....
60	14.50	25.00	156.0	41.60	39.60
70	.....	.....	178.5	.....	41.70
80	.....	.....	178.5	50.00	49.00
90	.....	.....	178.5	.....	50.00
100	.....	.....	.....	.....	50.00
120	14.50	25.00	.....	.....	.....

found to be inconvenient so other organic compounds were tried. The most useful one was a mixture of paraffin dissolved in xylene. This combination has no effect on a Nesslerized solution.

Although recovery experiments are not absolute proofs of the accuracy of a method, they are helpful. Table IV shows that all of the methods tested recovered practically all of the ammonia added.

COMPARISON OF METHODS

After becoming familiar with the various methods, they were used as nearly simultaneously as possible on juices from different plant species. The results obtained by the methods are given in table V. For convenience in comparison FOLIN'S method was arbitrarily chosen as the standard. The relationships are determined in the following manner. The individual results in any one column and the corresponding results by FOLIN'S permutit method are averaged separately and the relationship between the two is calculated, using the average obtained by the FOLIN method as a base of 100.

An examination of table V indicates the relative values to be expected when any of the methods are used. The FOLIN and aspiration methods give closely comparable results; the modified permutit and VAN SLYKE methods tend toward lower results which are also comparable; while those obtained by VICKERY and PUCHER'S method are high. The differences between the methods are probably within the errors of sampling, so that any one of the tested procedures can be used. It appears, however, that VICKERY and PUCHER'S technique as applied to expressed plant juice probably approaches the limits within which these procedures are applicable.

TABLE IV

PERCENTAGE OF RECOVERY OF AMMONIA NITROGEN ADDED TO PLANT JUICE BY VARIOUS METHODS

FOLIN PERMUTIT		AERATION				VICKERY AND PUCHER				VAN SLYKE	
CELERY	BEETS	CELERY		BEETS		CELERY		BEETS		CELERY	BEETS
NESSLER	NESSLER	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	NESSLER
103.0	95.0	101.0	102.0	92.6	96.5	105.0	99.3	99.7	.....	101.0	100.0
98.5	94.3	103.0	98.5	97.2	96.4	98.4	96.8	97.0	.....	101.0	97.9
103.0	96.0	103.0	107.0	96.9	.....	99.0	93.5	99.0	.....	95.0	97.9
101.0	.....	100.0	97.0	103.7	.....	98.5	101.5	100.5	.....	98.0	.....
.....	.....	97.5	99.0	103.0	.....	98.5	.....	99.5	.....	.....	.....
.....	.....	99.0	.....	103.2	.....	.....	.....	100.0	.....	.....	.....
.....	.....	101.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average ... 101.3	95.1	100.6	100.7	99.5	96.4	99.9	98.0	99.2	.....	98.7	98.6

TABLE V

COMPARISON OF AMMONIA NITROGEN IN PLANT JUICE AS DETERMINED BY VARIOUS METHODS

		FOLIN	MODIFIED PERMUTIT		ASPIRATION		VICKERY AND PUCHER		VAN SLYKE
		NESSLER	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Beets	1	26.7	.....	.....	.....	24.9	26.7	27.4	.....
	2	6.8	.....	.....	.....	.....	6.9	9.5	.....
	3	6.0	.....	.....	.....	.....	6.1	10.9	.....
	4	6.5	.....	.....	.....	.....	6.5	.....	.....
	5	19.5	.....	.....	.....	24.1	20.0	23.5	.....
	6	10.3	.....	.....	.....	9.8	10.5	13.7	.....
Celery	7	10.0	.....	.....	13.3	14.0	17.0	14.8	.....
	8	13.8	.....	.....	16.7	17.1	19.0	19.6	.....
	9	13.3	.....	.....	13.2	14.8	15.1	20.0	.....
	10	16.7	.....	.....	17.0	15.4	22.0	26.0	.....
	11	11.1	.....	.....	15.8	15.1	24.0	20.5	.....
	12	12.5	.....	.....	12.5	9.0	15.7	13.4	.....
	13	11.5	.....	.....	11.5	8.1	19.6	16.0	.....
Beets	14	14.9	14.3	14.3	15.9	14.3	19.8	18.5	15.0
	15	33.4	31.2	.....	33.4	.....	35.3	.....	32.9
	16	80.0	75.8	74.8	84.8	79.1	86.2	86.5	73.5
Celery	17	40.3	37.8	35.3	40.2	44.8	45.5	46.8	24.5
	18	80.6*	57.1	.....	55.5	.....	62.5	.....	55.6
Spinach	19	50.0	47.0	45.7	47.0	46.6	51.2	53.0	47.5
	20	80.0	78.3	.....	78.3	.....	83.5	.....	74.8
	21	57.6	53.0	.....	52.5	.....	62.5	.....	54.3
	22	44.2	37.8	31.4	39.3	39.5	48.5	50.0	41.0
Average p.p.m.		50.0	46.9	.....	48.3	.....	53.9	.....	46.7
Percentage of FOLIN method		100.0	94.0	88.2	100.4	100.4	110.6	121.0	90.7

* This result is much too high, owing to the amount of interfering yellow pigment. In all cases it and the corresponding values in the other columns have been left out of the calculations of relationship of the other methods to the FOLIN method.

Discussion

The results in table V are shown relatively, with no concern as to their accuracy for plant use. Of all the methods described, it is the writer's belief that FOLIN'S permutit method, as modified in this investigation, is the most accurate. If any of the usual distillation or aeration methods are allowed to proceed, the evolution of ammonia continues indefinitely. This makes it necessary to add a time factor to these procedures to avoid the inclusion of ammonia due to the hydrolysis of metabolized nitrogen. This difficulty is overcome by the use of permutit followed by aeration. The permutit removes an amount of ammonia which corresponds to that given off up to the time when there is a break in the time-concentration curve (fig. 1), and subsequent aeration removes the ammonia free from any

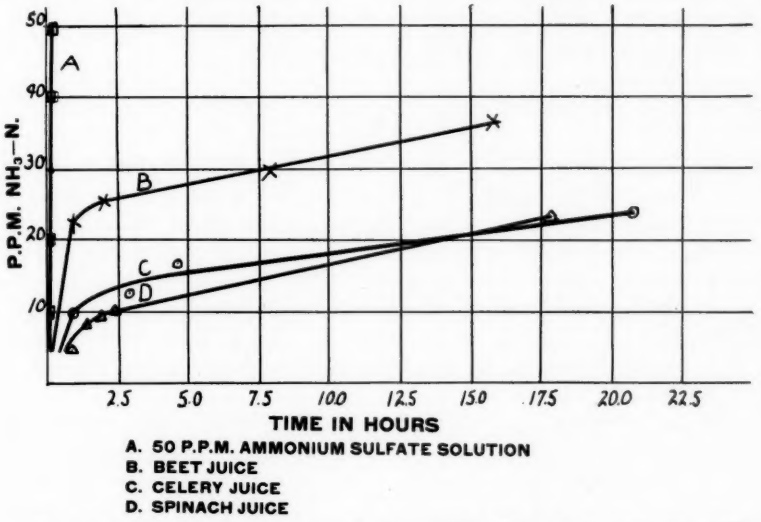

FIG. 1. Graph representing amount of ammonia nitrogen, in parts per million, aerated with time.

substance which interferes with Nesslerization. There is, however, a possible source of error in this method. As stated before, FOLIN has shown that in the presence of a certain salt concentration permutit does not function perfectly, and it is possible that a juice may have this inorganic salt concentration, thus inhibiting its use. The inorganic salt concentrations of the juices used in this study lend themselves to the use of permutit. For expressed juices which contain prohibitive amounts of inorganic salts, dilution may be used to overcome this factor.

Summary and conclusions

1. Five methods for the determination of ammonia have been compared, all of which give rather close agreement.

2. FOLIN'S permutit method readily adsorbed not only ammonia from plant juice but also a yellow pigment. This pigment, like ammonia, was released from permutit upon the addition of sodium hydroxide.

3. A new procedure combining the use of permutit and aeration gives reliable results and overcomes the difficulties of adsorbed pigments and hydrolyzable substances.

4. All methods recovered known amounts of added ammonia quantitatively.

The writer wishes to express his appreciation to Mr. JOHN B. SMITH for his assistance during the course of this investigation.

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MANGANESE ESSENTIAL FOR THE GROWTH OF *LEMNA MAJOR*[1]

J. S. McHargue and R. K. Calfee

(WITH THREE FIGURES)

Previous reports (**3–8**) from this laboratory have shown that manganese is an essential element for the normal growth and maturation of important agricultural crops, and that it has an important function in the synthesis of chlorophyll. Our experiments were carried on in both water and purified sand cultures. In our former reports attention was called to the more common sources of manganese contamination and the necessity of eliminating traces of this element from plant nutrients, distilled water, and vessels in which plants are to be grown.

In our previous work, experiments to show the necessity of manganese for plant growth were made with land plants which have some economic importance and a much more complex morphological structure than *Lemna major*.

Lemna major (*Spirodela polyrhiza*) is a minute, stemless, free-floating, aquatic plant consisting of a mere frond from which, on the under side, grow roots about 10 mm. long. The plants propagate by the proliferous growth of a new individual from a cleft in the edge or base of the parent frond, and also by autumnal fronds in the form of minute bulblets which sink to the bottom of the water during the winter season but rise to the top and vegetate in the spring.

Clark and Fly (**1**) have recently stated that manganese is not necessary for the growth of *Lemna major*. Because this plant synthesizes chlorophyll, we became doubtful of the findings of these investigators, and have carried on experiments for the past six months to ascertain to our satisfaction whether or not manganese is essential for the growth of this plant. Normal plants were collected from the surface of a pond about the middle of July, 1931, and transferred to water cultures contained in acid-proof stoneware jars. The solutions in which the plants were grown contained the following amounts of mineral plant nutrients:

Calcium nitrate, $Ca(NO_3)_2.5H_2O$	4.0 gm.
Potassium nitrate, KNO_3	1.0 "
Magnesium sulphate, $MgSO_4.7H_2O$	1.0 "

[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Potassium acid phosphate, KH_2PO_4	1.0 "
Potassium chloride, KCl	0.5 "
Ferric citrate, $FeC_6H_5O_7.3H_2O$	0.1 "
Distilled water	7 liters

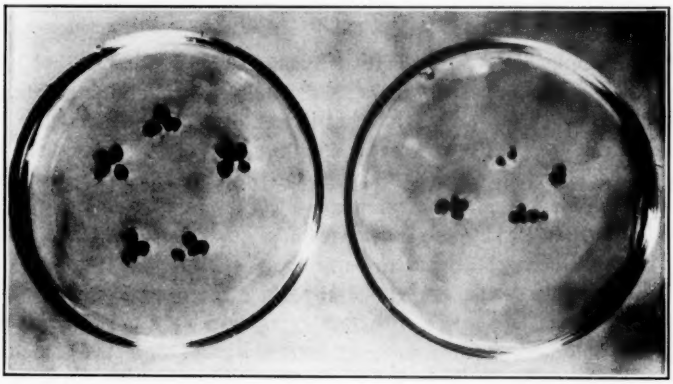

FIG. 1. Influence of manganese on the size and color of *Lemna major:* plants on left were grown in a solution containing 0.25 p.p.m. manganese; plants on right are manganese-deficient. 4/5 actual size.

The salts used in preparing this solution were tested chemically to ascertain their freedom from a number of the less common elements, manganese included. The water was distilled and condensed in a quartz tube, and collected and stored in an acid-proof stoneware receiver. To avoid possible complications from the absence of elements other than manganese, 0.5 part per million of boron, and 50 parts per billion each of copper and zinc were included in the manganese-free medium.

Plants from a stock culture of *Lemna* (containing manganese) were transferred to two jars of this medium, one of which had received enough manganese sulphate to bring the manganese content to 0.5 part per million but no manganese was added to the second jar. There was no discernible difference in the growth or appearance of the two cultures at the end of eight weeks. Plants in both cultures had practically covered the surface of the solutions. A few plants from each culture were then transferred to fresh solutions of corresponding composition. At the end of eight weeks more the appearance of the plants in the two cultures was practically identical, but the rate of growth was unequal, as evidenced by the somewhat crowded condition of the plants in the manganese-treated culture as compared with the more sparse growth of plants in the manganese-deficient culture. Transfers were again made to fresh solutions. Normal growth

occurred in the manganese-treated culture, the plants covering all available space in eight weeks. The plants in the manganese-deficient culture appeared to be normal for two weeks, but during the third week many of the fronds were notably lighter in color and smaller in size. Definite chlorosis was present in this culture by the end of the third week. Figure 1 compares the size and gives some indication of the difference in color of representative plants of the treated and deficient cultures. At the beginning of the fifth week several of the fronds were dead, necrotic areas were present on many others, and all were chlorotic. The living plants at this time were separated into two equal groups, forming two cultures. Ferric nitrate (0.0005 gm. per liter, manganese-free) was added to the first culture, but failed to relieve the condition, and the plants died. Manganese sulphate at the rate of 10 parts per billion was added to the second culture of chlorotic plants. Many plants in this culture, those that showed extreme chlorosis, died. The majority, however, slowly regained some of their original green color and within eight days had resumed growth and production of new fronds. This culture was reserved as a stock culture for further experiments. The volume was kept up by the addition of a manganese-free nutrient solution and traces of manganese were added at the first evidence of chlorosis. The rate of reproduction in this culture was considerably below that of the cultures containing an abundance of manganese.

To ascertain the influence of manganese on the reproduction of *Lemna*, two groups of six jars were started with one frond each, selected for uniformity of size and color, from the culture of low manganese content. The total number of fronds was counted at the end of each week, for eight weeks. The average of each group is shown in figure 2.

Because of the small increase in weight that manganese-deficient cultures made before death, it was impracticable to produce sufficient material for a complete chemical analysis. By greatly increasing the size of the stock cultures and by using a large number of fronds to start the manganese-deficient cultures, enough material to determine ash, manganese, and iron eventually was obtained. Abundance of material was available without difficulty from the rapid-growing manganese-treated cultures. The results are given in table I, calculated as percentages of the moisture-free plants.

The failure of *Lemna* to make normal growth in a nutrient solution free from manganese apparently was due to a disturbance of metabolism affecting the chlorophyll, resulting in chlorosis, followed by necrosis and death of the plant. The rate of reproduction was affected pronouncedly by the manganese content of the culture. This effect was more marked after several weeks of growth, largely because of the greater number of plants capable of producing new fronds in the manganese-treated cultures, and also because of the slightly higher rate of growth of the individual fronds. At the end

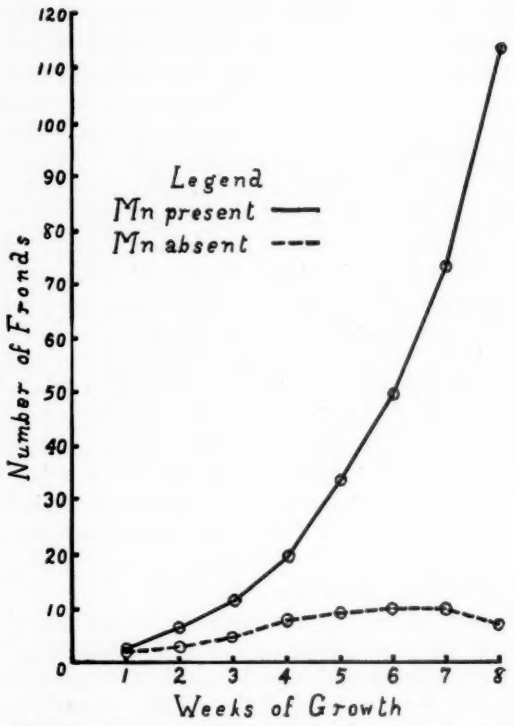

FIG. 2. Effect of manganese on rate of growth and reproduction of *Lemna major*.

TABLE I

ASH, MANGANESE, AND IRON CONTACT OF *Lemna* GROWN IN DIFFERENT MANGANESE CONCENTRATIONS

	MN, ABSENT (DEFICIENT)	MN, 7 P.P.B. (MAINTENANCE)	MN, 250 P.P.B. (OPTIMUM)
	per cent.	*per cent.*	*per cent.*
Ash	18.575	19.342	21.080
Mn	0.005	0.009	0.059
Fe	0.172	0.155	0.070

of the first week, the manganese-deficient cultures contained practically the same number of fronds as the manganese-treated cultures. At the end of the fourth week, the plants in the manganese-deficient cultures were chlorotic and reproduction had practically ceased. By the end of the sixth

week some of the fronds were dying. The nature of the plant made it impossible to determine whether this difference in the rate of reproduction was due directly to the absence of manganese or to the starved condition of the plant which resulted from manganese deficiency.

The total mineral content of the fronds was decreased by the absence of manganese in the culture. The manganese content of chlorotic and necrotic plants was less than one-tenth that of normal plants. The amount of manganese was small but was sufficient to be determined in a colorimeter. The iron content of the plants increased as the manganese decreased, suggesting an attempt at substitution on the part of the plant.

Investigations in this laboratory with *Lemna major* proved it to be rather unsatisfactory material for experimentation. Since it is a floating plant, the surface of the medium must be greater than the leaf surface produced, and as the fronds constitute practically the entire weight of the plant, the dry material obtained is much less than most experimental plants would produce on equivalent bench space. The plant consists essentially of roots and leaves, so evidence of deficiencies or toxicities must be obtained from the effects on size and color of the fronds, condition of the roots, and the rate of reproduction. Figure 3 shows the relative size and

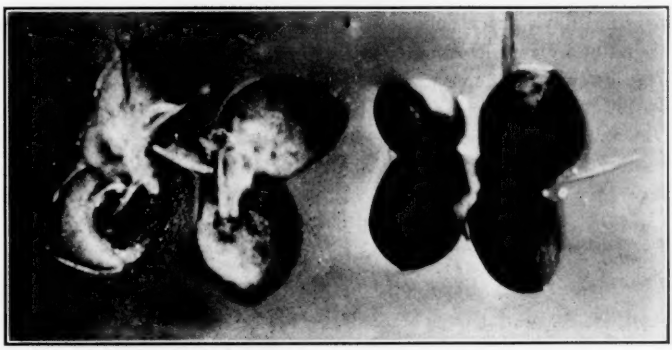

FIG. 3. *Lemna major:* left, under side showing roots and manner of producing new fronds; right, top view. Magnified about 4 times.

the parts of the plant. New fronds are produced from older fronds from buds, or special cells. This method of reproduction is not favorable for investigation of deficiencies that may influence the rate of reproduction.

Since our experiments with *Lemna major* were begun, HOPKINS (**2**) has published results showing that manganese is necessary for the growth of *L. minor*. Our results on *L. major*, although obtained by a somewhat different procedure, confirm his findings.

CLARK and FLY, in referring to previous work by the senior author (**8**), misquote him in a way that requires correction. The statement of CLARK and FLY is as follows: "He [MCHARGUE] suggests here that it is the manganese which causes the deep blue color of blue grass, etc." The writer in the article referred to makes this statement, however: "Kentucky bluegrass is particularly rich in manganese and chlorophyll and therefore affords an interesting example of the function of manganese in the development of the beautiful deep *green* color which is characteristic of the luxuriant bluegrass pastures." Doubtless many persons who have visited the bluegrass region of central Kentucky have wondered where the blue is in *Poa pratensis*. Incidentally, we commend the wisdom of the Committee on Standardized Plant Names, in making one word of bluegrass.

Summary

1. Fronds of *Lemna major* were grown in nutrient solution with and without manganese.

2. A deficiency of manganese in the nutrient solution resulted in retarded growth, failure to reproduce, chlorosis, necrosis, and death after utilization of the residual metal in the plants.

3. Chlorotic plants in manganese-deficient solutions were restored to their original dark green color by the addition of traces of manganese sulphate but not by ferric nitrate.

4. The exclusion of manganese from the medium resulted in a decrease in the total ash content and an increase in the iron content of the plant.

5. Because of its inherent characteristics, *Lemna* was found to be less satisfactory than most experimental plants for greenhouse cultures.

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CARBOHYDRATE CONTENT OF TOMATO FRUIT

L. G. SAYWELL and D. P. ROBERTSON

The carbohydrate content of mature tomato fruit has received considerable attention since ATWATER and BRYANT (**1**) indicated the total sugar content to be about 3.39 per cent. and the starch less than 0.1 per cent. on the fresh basis. In a later publication of the same series containing revised data, CHATFIELD and ADAMS (**6**) gave the average reducing sugar content as 3.37 per cent. MYERS and CROLL (**11**) reported the reducing sugar content of two samples of tomatoes to be 3.39 and 2.91 per cent. and the total sugar content 3.47 and 3.25 per cent. BELL, LONG, and HILL (**2**) determined the available carbohydrate content of two samples to be 2.6 and 3.0 per cent., respectively. LAWRENCE and MCCANCE (**9**) have placed it at 2.4 per cent. ROSA (**13, 14**) has presented a study of the composition of tomatoes at different stages of maturity. For the mature fruit, the reducing sugar content of five samples varied from 2.63 to 3.80 per cent. For two of these the sucrose content, by invertase inversion, was 0.04 and 0.11 per cent. The starch content, by diastase inversion, varied from 0.012 to 0.52 per cent. for the five samples. For the same two samples upon which sucrose determinations were made the values for acid-hydrolyzable material, by hydrolysis with 5 per cent. hydrochloric acid, were 0.180 and 0.212 per cent. respectively. BENOY and WEBSTER (**3**) have reported that the total and reducing sugar concentrations were equal. They reported values of 2.43 per cent. for each, and 0.069 per cent. of starch on the fresh basis. BIGELOW and STEVENSON (**5**) and BIGELOW and FITZGERALD (**4**) have studied the chemical composition of commercial tomato products prepared in the eastern tomato-growing sections of the United States. Their values for reducing sugar content of filtrates from pulps averaged about 4.20 per cent. CRUESS, SAYWELL, and HARK (**8**) have given preliminary data on the composition of California tomatoes.

The present investigation was undertaken in order to secure a more complete knowledge of the carbohydrate composition of California tomatoes. The plan has been to study the composition of composite samples of both the entire fruit and the several more or less distinct botanical regions of the fruit. Determinations have been made of the reducing sugars, sucrose, starch, and acid-hydrolyzable material and the dextrose-levulose ratios.

Materials and methods

Large samples of from 15 to 20 previously frozen fruits were taken to reduce sampling error. Each fruit was quartered through the polar diameter and two alternate quarters were used for a composite sample. When it was desired to have samples of the core, locule, interlocular septa and outer walls,

the remaining quarters were cut through the equatorial diameter and the required portions then carefully secured. The samples were then ground in a food chopper and analyzed at once or were placed in cold storage (0° C.) if the analysis could not be completed immediately.

Reducing sugars were determined by the SHAFFER-HARTMAN method (**15**) after clarifying with saturated neutral lead acetate and deleading with sodium oxalate. Dextrose was determined by the method of LOTHROP and HOLMES (**10**). Sucrose was inverted with invertase for one hour at 55° C. The total reducing sugars present then were determined and the sucrose content calculated by difference. Starch was determined by the following modification of OLMSTED's method (**12**). To a 25-cc. aliquot of the sample 0.1 gram of Taka-diastase was added. After careful mixing, the solution was brought to 55° C. and held at this temperature for one hour. After cooling and clarifying, the reducing sugars were determined as previously described. The acid-hydrolyzable carbohydrate material was determined by direct acid hydrolysis (**16**). This fraction includes the starch. Proper blanks were made for all determinations.

Total solids were calculated from the refractive index-total solids ratio determined by CRUESS and SAYWELL (**7**) for California tomatoes.

Results and discussion

For five samples analyzed, the sucrose content was 0.03, 0.05, 0.04, 0.02, and 0.07 per cent. respectively. Since these values were fairly uniform and all less than 0.10 per cent. it appeared that the sucrose content of mature fruit was relatively small and frequently less than 0.05 per cent. Consequently further determinations were not made. However, it is not intended to preclude the possibility of important physiological functions of sucrose.

Starch determinations were made on the above five samples. Values of 0.045, 0.038, 0.040, 0.031, and 0.037 per cent. starch were obtained. The first two samples were from vines of the San Jose Canner variety, the third and fourth from Santa Clara Canner vines, and the fifth from Stone. ROSA (**13, 14**) has reported values of 0.044, 0.052, 0.022, 0.034, and 0.012 per cent. on samples of ripe fruit of Earliana, Globe, and Stone varieties. Since the two series of results agreed quite closely, it appeared that the usual range of starch content of mature fruit was from 0.02 to 0.05 per cent., expressed on the fresh basis.

In order to secure data on reducing sugars representative of the state, samples from the more important tomato producing districts were analyzed with the results given in table I.

It would appear from table I that the average reducing sugar content of California tomatoes is somewhat higher than that generally recorded in the literature for other localities. Considerable variation was found in the

TABLE I

REDUCING SUGAR CONTENT OF MATURE TOMATO FRUIT GROWN IN CALIFORNIA
(EXPRESSED AS THE PRECENTAGE OF THE FRESH WEIGHT)

LOCALITY	REDUCING SUGARS	TOTAL SOLIDS	LOCALITY	REDUCING SUGARS	TOTAL SOLIDS
Arlington	3.28	6.57	Locke	3.96	7.22
Buena Park	3.62	8.13	Riverside	2.95	6.05
Burbank	3.45	7.30	Sacramento	4.01	6.82
Centerville	4.00	8.05	Salinas	3.94	6.82
Chino	3.04	6.88	San Fernando	3.33	6.43
Davis	3.54	6.93	San Jose	3.66	6.92
El Monte	3.38	6.42	Santa Clara	3.38	6.30
Fullerton	3.57	7.41	Sunnyvale	3.80	7.03
Hayward	3.81	7.22	Average	3.57	6.97

total solids and reducing sugars of samples from different districts within the state.

The results of the analyses of the cores, the locules, the interlocular walls and outer wall portions of alternate quarters of the fruit and a corresponding composite of these regions prepared from the remaining quarters are given in table II. Samples were taken October 2, 1931.

From these data it is apparent that the tissue of the core region is relatively high in reducing sugars, total solids, and acid-hydrolyzable contents. The locular material is considerably lower in total solids, reducing sugars, and acid-hydrolyzable material than any other portion of the fruit. The acid-hydrolyzable content is very much lower in the locular material. The walls approach the core and composite in composition except that they are somewhat lower in reducing sugars.

The results of the dextrose-levulose and total solids determinations on representative samples from several of the important tomato producing regions of the state are given in table III. It is interesting to note that the dextrose-levulose ratios generally are quite uniform and that the average levulose content is about 46 per cent. of the average total reducing sugar content.

Summary

The sucrose content of California tomatoes varied from very small quantities up to 0.05 per cent. and the reducing sugar content, from 3.30 to 3.70 per cent.; the dextrose content was about 1.2 times the levulose content. The starch content varied from 0.01 to 0.06 per cent., while the acid-hydrolyzable material varied from 0.10 to 0.30 per cent. The total solids content ranged from 6.5 to 7.0 per cent. on the average. All results are expressed on the basis of the fresh weight of the fruit.

TABLE II

COMPOSITION OF DIFFERENT REGIONS OF TOMATO FRUIT (EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT)

SAMPLE NUMBER	REGION	REDUCING SUGARS	TOTAL SOLIDS	SAMPLE NUMBER	REGION	REDUCING SUGARS	TOTAL SOLIDS	ACID-HYDROLYZABLE
		per cent.	*per cent.*			*per cent.*	*per cent.*	*per cent.*
1–1	Core	2.90	7.1	2–4	Core	2.61	5.2	——
	Locule	2.69	6.6		Locule	2.13	5.0	——
	Walls	2.32	6.6		Walls	2.38	5.0	——
	Composite	2.70	6.6		Composite	2.48	5.0	——
1–2	Core	3.30	6.3	3–2	Core	3.47	5.2	——
	Locule	2.90	6.0		Locule	3.15	5.1	——
	Walls	2.96	6.2		Walls	3.76	5.1	——
	Composite	3.08	6.2		Composite	3.67	5.2	——
2–1	Core	3.18	6.2	1–1–2	Core	3.61	6.3	0.245
	Locule	2.72	5.9		Locule	3.26	6.2	0.055
	Walls	3.04	6.2		Walls	3.30	6.2	0.265
	Composite	3.10	6.2		Composite	3.49	6.2	0.210
2–2	Core	2.60	5.8	1–2–2	Core	2.77	6.3	0.320
	Locule	2.13	5.1		Locule	2.30	5.8	0.060
	Walls	2.37	5.5		Walls	2.85	6.2	0.240
	Composite	2.49	5.6		Composite	2.96	6.2	0.235
1–3	Core	2.94	6.2	Marglobe (1)	Core	3.05	7.1	0.260
	Locule	2.52	5.8		Locule	2.53	7.2	0.065
	Walls	2.79	5.8		Walls	2.73	7.2	0.282
	Composite	2.87	5.9		Composite	2.92	7.2	0.275
1–4	Core	2.66	6.0	Santa Clara (3)	Core	3.30	5.1	0.210
	Locule	2.07	6.0		Locule	2.98	5.0	0.050
	Walls	2.53	5.8		Walls	3.07	5.0	0.370
	Composite	2.62	5.9		Composite	3.23	5.1	0.320
1–5	Core	3.36	6.3	Santa Clara (2)	Composite	2.67	5.1	0.295
	Locule	2.86	6.2	Marglobe (2)	Composite	3.92	7.2	0.260
	Walls	2.84	6.0	Marglobe (3)	Composite	3.93	7.2	0.295
	Composite	3.13	6.2	Bonnie Best	Composite	3.76	7.8	0.265
2–2	Core	2.86	5.0	Average	Core	3.06	6.1	0.259
	Locule	2.34	4.9	"	Locule	2.63	5.8	0.057
	Walls	2.39	5.0	"	Walls	2.84	5.9	0.289
	Composite	2.58	5.0	"	Composite	3.12	6.1	0.269

TABLE III

DEXTROSE AND LEVULOSE CONTENT OF MATURE TOMATO FRUITS
(EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT)

LOCALITY	DEXTROSE	LEVULOSE	RATIO D/L	TOTAL SOLIDS
	per cent.	*per cent.*	*per cent.*	*per cent.*
Burbank	2.06	1.85	1.11	6.9
Chino	2.06	1.71	1.20	7.1
San Fernando	1.93	1.73	1.11	6.6
San Fernando	2.11	1.68	1.25	6.9
San Fernando	2.04	1.81	1.13	6.4
Burbank	2.15	1.84	1.17	6.5
Burbank	2.06	1.92	1.07	6.3
Arlington	1.99	1.91	1.04	6.3
Arlington	1.98	1.92	1.03	6.4
El Monte	2.09	1.97	1.06	6.4
El Monte	1.89	1.59	1.19	6.2
Fruitvale	2.50	2.04	1.22	8.8
Fruitvale	2.37	1.97	1.20	8.4
San Jose	2.35	1.81	1.30	8.1
San Jose	2.24	1.73	1.29	7.9
Isleton	2.39	1.96	1.22	8.5
Palo Alto	2.12	1.78	1.19	8.1
Average	2.14	1.83	1.18	7.2

The average sugar content of tomatoes grown in this state appears to be somewhat higher than that previously reported in the literature.

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RELATION BETWEEN THE GREEN AND THE YELLOW PIGMENTS IN CHLOROTIC LEAVES

(WITH ONE FIGURE)

J. OSERKOWSKY

WILLSTÄTTER and STOLL (**13**) observed that the ratio of green to yellow pigments in green leaves was more or less constant for various plants. LUBIMENKO (**4**) found that the ratio of chlorophyll to carotinoids in many plants varied only a little, but a wider variation was observed between old and young leaves of the same plant; this ratio being, on the whole, larger in the leaves which were richer in chlorophyll. Despite this variation, the data of LUBIMENKO reveal a close relation between the amount of the green and yellow pigments in leaves of different ages. This relation may be approximated by a linear equation. SPRAGUE and SHIVE (**12**) and SCHERTZ (**8, 9**) determined the chlorophyll content and the yellow pigments in leaves collected at various times during the season. Their data indicate that no consistent quantitative relation exists between the green and the yellow pigments, although in several series of samples a more or less close correlation was found.

A close parallelism between the development of the green and yellow pigments has been observed in etiolated plants exposed to light. ELFVING (**1**) and KOHL (**3**) claimed that the yellow pigments increase in etiolated plants exposed to light. They did not, however, determine the amount of chlorophyll formed during exposure. EULER (**2**) and SJÖBERG (**10**) studied this process quantitatively, and found that on illumination of etiolated seedlings the green and the yellow pigments increase simultaneously.

Chlorosis[1] is usually looked upon as a deficiency of chlorophyll only. Chlorotic leaves are yellow, although severely chlorotic leaves may be a pale cream color or white. Aside from casual observations as to the color of chlorotic leaves, no quantitative data are available in the literature concerning the amount of yellow pigments in chlorotic leaves. The present work was undertaken to ascertain whether there exists a correlation between the amount of yellow pigments and chlorophyll in chlorotic leaves.

The material used in the present investigation was gathered from trees affected with lime-induced chlorosis which was found to be curable by administration of iron salts to the trees. The term "green leaves from treated trees" in table I refers to green leaves from chlorotic trees which became green as a result of treatment with iron salts. Fresh leaves only

[1] The term chlorosis as used in this paper refers only to chlorosis curable by iron salts.

were used for the analysis of the leaf pigments. The pigments were determined colorimetrically, according to the method of WILLSTÄTTER and STOLL (**14**) as modified by SCHERTZ (**7**). The green color of the potassium chlorophyllin was matched against a similar solution prepared from pure chlorophyll, obtained from fresh green grass by the method of WILLSTÄTTER and STOLL (**13,** pp. 38–41). The yellow pigments in the ether solution were determined colorimetrically against a solution of 0.2 per cent. $K_2Cr_2O_7$, and the results were calculated as "xanthophyll," assuming that all the yellow pigments in the leaves were present as xanthophyll. The calculation was carried out according to the data given by WILLSTÄTTER and STOLL (**14,** p. 25).

The methods used in the present work may be open to criticism for the following reasons: The ratio of chlorophyll *a* to chlorophyll *b* may not be the same in green and chlorotic leaves (**6**), and may not be that of the standard chlorophyllin solution. Also the assumption that all the yellow pigment in the leaves was xanthophyll is obviously inaccurate. It is believed, nevertheless, that the data thus obtained afford a means of judging the relative amounts of the yellow pigments present in leaves. It may be noted that the data of WILLSTÄTTER and STOLL (**13, 14**) indicate that xanthophyll predominates over the carotin in green as well as in yellow leaves. Similar results were obtained also by other investigators. The results of the analyses are presented in table I. In figure 1 are plotted the data for Hardy pear leaves only. The data in column 7, table I, show that the ratio of chlorophyll to the yellow pigments is not constant and is smaller in leaves containing the smaller amounts of chlorophyll. But despite the inaccuracies involved in the analytical methods used, the results obtained show a marked positive correlation between the chlorophyll content and the amount of yellow pigments in chlorotic leaves, and in green leaves from chlorotic trees or from trees treated with iron. The coefficient of correlation of chlorophyll to xanthophyll for 31 samples of Hardy pear leaves was found to be 0.934. *Chlorotic leaves are thus deficient in yellow pigments as well as in chlorophyll.* This relation is similar to that existing in etiolated plants. None of the samples analyzed was free from chlorophyll. It is impossible, therefore, to conclude from the available data whether chlorotic leaves devoid of chlorophyll are also devoid of yellow pigments.

Various hypotheses were advanced to explain the more or less constant ratio between the yellow and the green pigments in leaves, which was observed in several instances. LUBIMENKO (**4**) suggested that the yellow and the green pigments of the leaves were derived from a common substance; while SMITH (**11**) concluded from the similarity between the constitution of phytol, a component of chlorophyll, and the carotinoids, that

TABLE I

CHLOROPHYLL AND YELLOW PIGMENTS (REPORTED AS XANTHOPHYLL) IN GREEN AND CHLOROTIC LEAVES

TREES FROM WHICH SAMPLES WERE COLLECTED	PARTS OF TREES FROM WHICH SAMPLES WERE COLLECTED	DESCRIPTION OF LEAVES	DATE OF COLLECTION	PER CENT. OF DRY WEIGHT		RATIO CHLOROPHYLL/XANTHOPHYLL
				CHLOROPHYLL CONTENT OF LEAVES	XANTHOPHYLL CONTENT OF LEAVES	
Hardy pear	Spurs	Moderately chlorotic	May 27	0.15	0.052	2.9
"	"	Green leaves from treated trees	"	0.78	0.15	5.2
"	"	Moderately chlorotic	June 13	0.12	0.064	1.9
"	"	Severely chlorotic	June 13	0.061	0.032	1.9
"	"	Moderately chlorotic	"	0.18	0.058	3.1
"	"	Green from chlorotic trees	"	0.40	0.083	4.8
"	"	Green from treated trees	"	0.92	0.17	5.4
"	"	Green from treated trees	"	0.78	0.17	4.6
"	"	Severely chlorotic	July 9	0.061	0.028	2.2
"	"	Moderately chlorotic	"	0.18	0.072	2.5
"	"	Green from chlorotic trees	"	0.44	0.10	4.4
"	"	Green from treated trees	"	0.73	0.14	5.2
"	Base of shoot	Severely chlorotic	"	0.071	0.036	2.0
"	"	Slight green from chlorotic trees	"	0.29	0.076	5.1
"	"	Deep green from chlorotic trees	"	0.67	0.15	4.5
"	"	Deep green from treated trees	"	0.77	0.18	4.3
"	Terminal end of shoot	Green from chlorotic trees	"	0.60	0.14	4.3
"	Spurs	Severely chlorotic	July 20	0.10	0.044	2.3
"	"	Moderately chlorotic	"	0.26	0.074	3.5
"	"	Slight green from chlorotic trees	"	0.43	0.13	3.3
"	"	Deep green from chlorotic trees	"	0.96	0.13	7.4

TABLE I—(*Continued*)

Trees from which samples were collected	Parts of trees from which samples were collected	Description of leaves	Date of collection	Per cent. of dry weight		Ratio chlorophyll/xanthophyll
				Chlorophyll content of leaves	Xanthophyll content of leaves	
Hardy pear	Spurs	Severely chlorotic	Aug. 8	0.057	0.041	1.4
"	"	Moderately chlorotic	"	0.15	0.060	2.5
"	"	Slight green from chlorotic trees	"	0.38	0.10	3.8
"	"	Deep green from chlorotic trees	"	0.65	0.16	4.1
"	"	Deep green from chlorotic trees	"	0.73	0.15	4.9
"	"	Severely chlorotic	Sept. 10	0.081	0.054	1.5
"	"	Moderately chlorotic	"	0.16	0.90	1.8
"	"	Slight green from chlorotic trees	"	0.38	0.10	3.8
"	"	Deep green from chlorotic trees	"	0.66	0.15	4.4
"	"	Deep green from treated trees	"	0.92	0.17	5.4
Bartlett pear	Terminal end of shoots	Severely chlorotic	July 20	0.087	0.047	1.8
"	"	Moderately chlorotic	"	0.27	0.089	3.0
"	"	Slight green from chlorotic trees	"	0.42	0.11	3.8
"	"	Deep green from chlorotic trees	"	0.68	0.12	5.7
Peach	Middle of shoots	Severely chlorotic	"	0.21	0.15	1.4
"	"	Moderately chlorotic	"	0.68	0.20	3.4
"	"	Slight green from chlorotic trees	"	1.10	0.21	5.2
"	"	Deep green from chlorotic trees	"	1.5	0.32	4.7

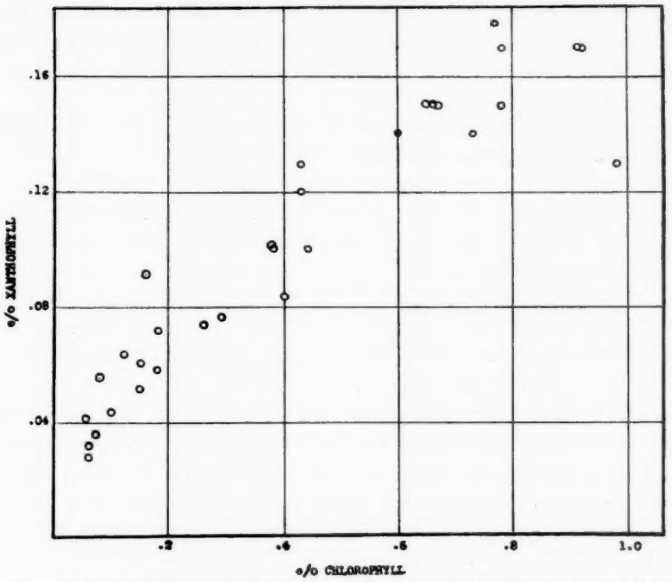

FIG. 1. Yellow pigments (reported as xanthophyll) and chlorophyll in Hardy pear leaves.

"it therefore seems probable that phytol either comes from the carotinoids, forms the carotinoids, or is produced from the same parent substance. In this way the constant proportionality existent between yellow and green pigments of the leaf may be explained" (p. 158).

There is at present no conclusive evidence for any of the hypotheses here mentioned. While any one of them may account for the results reported in this paper, it seems not unlikely that the "active iron" (5), which is the iron compound active in chlorophyll formation, may play a rôle in the formation of yellow leaf pigments.

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A MICRO-METHOD FOR NITROGEN IN PLANT MATERIAL

L. D. Doneen

Microchemical methods are often desirable or essential for plant nutrition studies. In a recent extensive investigation on nutrition of wheat, samples sufficiently large to determine certain nitrogen fractions of wheat in the seedling and early part of the stooling stage by the usual method were not available, and it became necessary to develop a suitable micro-method.

A review of the literature in the medical field indicated that one of the outstanding methods for determining small amounts of nitrogen is that developed by Folin and Denis (**2, 3, 4, 5, 6**), and later modified by Folin and Wu (**7**). This method, however, like others reviewed, did not include the fixation of nitrate nitrogen, which is necessary, especially with young plants containing large amounts of nitrate nitrogen. The use of oxidizing agents to promote digestion of organic materials is not new, and is practical for rapid digestion of organic material in the determination of nitrogen (**9, 10, 11, 14**). In some of the more recent works (**1, 8, 9**) protective colloids have been used to stabilize the color obtained by Nesslerization, thus preventing its precipitation with other compounds.

The proposed method involves the use of perchloric acid as an oxidizing agent to aid in the digestion process, and includes the addition of gum arabic as a protective colloid and the conversion of nitrate nitrogen to ammonia.

Procedure.—Place a sample of the plant juice, containing about 1 mg. of nitrogen, in a large test-tube (2.5 × 20 cm.) and evaporate to dryness. (If dry plant material is used, weigh out an equivalent amount.) Add 1 cc. sulphuric acid (nitrogen-free) containing 1 gm. of salicylic acid to 20 cc. of sulphuric acid. Mix thoroughly and allow to stand at least 20 minutes. If large quantities of organic material are necessary to obtain sufficient quantities of nitrogen for Nesslerization, 2 cc. of the sulphuric-salicylic acid mixture may be required. Add approximately 0.3 gm. of sodium thiosulphate and heat the solution gently until fumes appear at the mouth of the test-tube. Cool and add 1 cc. of 60 per cent. perchloric acid to which has been added 0.5 gm. of copper sulphate to 500 cc. of the acid. Digest over covered flame or micro-burner, heating very gently until foaming ceases, and digest at least two minutes after clearing. Usually the digestion mixture becomes clear in five minutes or less.

Transfer the digested mixture to a 100-cc. volumetric flask by washing the test-tube with distilled water and dilute to approximately 50 cc. Add 5 cc. of a 5 per cent. gum arabic solution, and neutralize with 10 per cent. sodium hydroxide. Ordinarily the gum arabic may be used without special

preparation, but if color is obtained upon treating it with Nessler's solution, or if reduction to free mercury occurs upon standing for some time, it is necessary to treat the gum arabic as directed by CHILES (**1**).

The results of a series of determinations on identical samples by the proposed micro-method, and the KJELDAHL-GUNNING method modified to include nitrate nitrogen, are given in table I. Samples no. 1 and no. 2 are

TABLE I

TOTAL NITROGEN DETERMINED ON PLANT EXTRACTS BY MICRO-METHOD AS COMPARED TO KJELDAHL METHOD

GREEN PLANT TISSUE	SAMPLE NO.	N PER CC.			DIFFERENCE
		MICRO.	AVE. MICRO.	KJELDAHL	
		mg.	*mg.*	*mg.*	*per cent.*
Chrysanthemum leaves, water-soluble fraction	1	0.2745	0.2715	0.2706	+ 0.33
	1	0.2715			
	1	0.2697			
	1	0.2705			
Young wheat plants, water-soluble fraction	2	0.2970	0.2975	0.3057	− 2.68
	2	0.2982			
	2	0.2940			
	2	0.3010			
Young wheat plants, expressed juice	3	1.886	1.874	1.865	+ 0.48
	3	1.851			
	3	1.886			
Seedling wheat plants, expressed juice	4	1.253	1.245	1.246	− 0.08
	4	1.254			
	4	1.245			
	4	1.230			
Bluegrass, expressed juice	5	0.555	0.555	0.565	− 1.76
	5	0.553			
	5	0.551			
	5	0.561			
Cineraria leaves, expressed juice	6	0.819	0.825	0.842	− 2.01
	6	0.831			

extract nitrogen or the water-soluble protein nitrogen as obtained by the method of NIGHTINGALE *et al.* (**12, 13**). The other samples represent expressed plant juice. Young plant tissue frequently contains appreciable quantities of nitrate nitrogen, and the wheat plants used in this experiment were known to contain large amounts of it. The results obtained by this colorimetric micro-method check closely with those of the modified KJELDAHL-GUNNING method. Therefore, the proposed method can be used satis-

factorily to supplement the KJELDAHL method when only small samples of plant materials are available for the determination of various nitrogen fractions, including nitrate nitrogen.

The efficiency in converting nitrate nitrogen to ammonia by the proposed method was further determined by additions of nitrate nitrogen to solutions and to plant material. The results given in table II show that approxi-

TABLE II

RECOVERY OF NITRATE NITROGEN ADDED TO WATER AND TO PLANT MATERIAL

SAMPLE	N IN SAMPLE	N ADDED AS NITRATES	TOTAL N DETERMINED	NITRATE NITROGEN RECOVERED	NITRATE N RECOVERED
	mg.	*mg.*	*mg.*	*mg.*	*per cent.*
Distilled water	None	0.0	0.000	0.000	0.0
	"	1.0	0.980	0.980	98.0
	"	"	0.973	0.973	97.3
	"	"	0.984	0.984	98.4
Wheat plant, expressed juice	1.245	0.2	1.444	0.199	99.5
	"	"	1.438	0.193	96.5
	"	"	1.440	0.195	97.5

mately 98 per cent. of the nitrate nitrogen added was recovered. It is reasonable to assume, therefore, that nitrate nitrogen naturally present in plant material would be converted likewise.

The method is rapid, since digestion is usually complete in less than 10 minutes. The writer has been able to complete about fifty digestions in an hour by having a number of samples digesting simultaneously.

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STUDIES OF THE HARDINESS OF PLANTS: A MODIFICATION OF THE NEWTON PRESSURE METHOD FOR SMALL SAMPLES[1]

S. T. DEXTER[2]

Introduction

In studying the hardiness of winter wheat, NEWTON (**7**) outlined a practical test for this quality which involved the measurement of the volume of juice expressed from the hardened leaves of the several varieties. He found that the hardier varieties were more resistant to the pressure treatment and gave a smaller volume of expressed juice than the tenderer ones. MARTIN (**3**) repeated the work, confirming NEWTON's results in a general way, but finding difficulty in selecting suitable samples. Freezing and subsequent wilting of portions of the plants resulted in a lack of uniformity of samples from day to day. Since rather considerable quantities of leaves were required for pressing, it was found to be impracticable to attempt the selection of uninjured tissue. MEYER (**5**), working with pitch pine needles, obtained similar results, while STEINMETZ (**8**) was unable to find any relation between press juice volume and hardiness in two varieties of alfalfa. STEINMETZ, however, froze the alfalfa roots with solid carbon dioxide (about $-80°$ C.) and ground them before pressing.

This paper is devoted largely to the description of a modification of the NEWTON method, which permits the experimenter to use very small samples which may be carefully selected to avoid imperfect leaves. The samples may consist of leaves from single plants, if individual differences are being studied. The weighed sample of leaf blades is placed upon a sheet (or several sheets) of ashless filter paper, so cut that thin layers of the leaves may be folded between sheets of the paper. The circular pad resulting is of such a size that it fits the small press cylinder to be used. The wrapped sample is placed within a washed, sheet-rubber flap to prevent possible contamination or loss of sap. To avoid irregularities of pressure, a pad of several inches of sponge rubber is placed in the bottom of the cylinder. Upon this pad the wrapped sample is placed. Below the piston (above the sample), a large rubber stopper which fits the cylinder is inserted in order to equalize the pressure on top of the sample. Suitable pressures are now applied for a short interval. It is found that the leaves are rarely, if ever, torn or broken within the pad when these precautions are observed.

[1] Contribution from the Hull Botanical Laboratory, University of Chicago.

[2] National Research Council Fellow.

Since the samples, as from individual plants, are small it is impracticable to try to measure the volumes of expressed sap as was done by NEWTON. In the modification proposed, the juice is taken up completely by the filter paper pads between which the leaves are pressed. The amount of sap expressed is measured in the following way. The unfolded pads and the residual pressed leaves are inserted into test-tubes, and a specified volume of distilled water is added. The sap solutes diffuse from the paper pad into this water. The more mineral matter that is so extracted, the higher will be the electrical conductivity of the water solution. NEWTON (**6**) has shown that the saps of the various varieties do not differ materially in electrical conductivity. In this way, therefore, one is able to estimate the relative amounts of sap expressed, even though the volumes are very small. There is, however, an additional feature. The leaves of the tender varieties are more bruised, or crushed, by this pressing process than are those of hardy varieties (MARTIN **3**). The exosmosis of minerals from such injured tissue might normally be expected to be more or less in proportion to such injury, that is, greater in the case of tender leaves and less in the case of hardy ones. This was found to be the case, even when no clear-cut differences in bruising could be distinguished visually.

The method, as outlined, was repeatedly applied in several ways,—with different sizes of samples, several pressures (66 to 660 atmospheres) and durations of pressures (1 to 5 minutes), and several absorption materials (old linen, cellu-cotton, filter paper). In every case the order of the varieties was correctly indicated, and the tenderer varieties showed higher electrical conductivities than the hardier ones. Measurements made with unhardened tissue did not show this characteristic difference.

Experimental results

On December 1, six 1-gm. samples of perfect leaf blades of Wis. Ped. no. 2 and of Minhardi winter wheat were prepared from plants growing in the field. Each sample was folded into a sheet of ashless filter paper weighing about 1 gm. (Whatman no. 42, 12.5 cm.), placed within a protecting rubber sheet, and subjected to a suddenly applied pressure. Duplicate samples of each variety were pressed at 1000, 2000, and 3000 pounds per square inch respectively. Each sample was pressed in turn, the firmly compacted pad was removed from the press cylinder, completely unfolded, and both paper and sample placed in a $1 \times 8''$ pyrex test-tube. The tubes were placed in a thermostatically regulated water bath at 2° C., and 25 cc. of distilled water at 2° C. were added to each tube, to cover the sample completely. Conductivity measurements were made after exosmosis had continued for the periods indicated in table I.

TABLE I

SPECIFIC CONDUCTIVITY ($\times 10^7$) (2° C.) IN RECIPROCAL OHMS OF AQUEOUS EXTRACTS OF PRESSED WHEAT LEAVES AND THE INCLOSING PADS; MINHARDI AND WISCONSIN PED. 2 VARIETY

PRESSURE	VARIETY	PERIOD OF EXOSMOSIS		
		2 HOURS	6 HOURS	18 HOURS
1000	Minhardi	326	409	549
	Wis. Ped. 2	463	566	799
2000	Minhardi	440	569	740
	Wis. Ped. 2	670	809	1060
3000	Minhardi	571	729	974
	Wis. Ped. 2	774	1117	1343

Minhardi is recognized as a very hardy variety, in field tests, and Wis. Ped. 2 as a hardy variety. From table I one may see that more mineral is obtained, in each case, from the tenderer variety. The injury from the pressing increases with both varieties, with increase in pressure.

On November 15, a similar experiment was run, using duplicate 1-gm. samples of leaves from three varieties of winter wheat, and a pressure of 10,000 pounds per square inch for one minute. The variety, Honor, is a medium hardy variety, tenderer than Wis. Ped. 2. Table II shows the results of this test. Conductivity readings of blanks on unpressed leaves

TABLE II

SPECIFIC CONDUCTIVITY ($\times 10^7$) (2° C.) IN RECIPROCAL OHMS OF AQUEOUS EXTRACTS OF PRESSED WHEAT LEAVES AND THE INCLOSING PADS. RESULTS OF EXOSMOSIS FROM FROZEN LEAVES ARE INCLUDED FOR COMPARISON

VARIETY	PERIOD FOR EXOSMOSIS				
	UNTREATED TISSUES	PRESSED 10,000 LB. PER SQUARE INCH			FROZEN −10° C., 2 HOURS
	2 HOURS	2 HOURS	6 HOURS	20 HOURS	4 HOURS
Minhardi	84	376	486	770	1368
Wis. Ped. 2	81	597	747	1178	2048
Honor	77	623	889	1316	2200
Filter paper	.....	.....	.....	43	.....

and on filter paper are included, as well as the results from exosmosis of leaves frozen at – 10° C. for 2 hours (DEXTER *et al.* **1, 2**).

The same relations of hardiness and resistance to pressure extraction of sap are seen in this table as in table I. It is interesting to note that the freezing-exosmosis method of DEXTER *et al.* gives conductivity values almost precisely proportional, for the three varieties, to those from the modified NEWTON method. (See also table III.)

In table III are shown the results of an experiment, performed November 27, in which the paper pads and the residual pressed leaves were separately extracted. Trumbull, a somewhat tender variety, and Minhardi were used. A pressure of 3000 pounds per square inch was applied for one minute. Conductivity values of extracts from samples frozen at –10° C. for two hours, on the same day, are included for comparison.

TABLE III

SPECIFIC CONDUCTIVITY ($\times 10^7$) (2° C.) IN RECIPROCAL OHMS OF AQUEOUS EXTRACTS OF PAPER PADS ALONE, AND OF PRESSED LEAF TISSUE OF WINTER WHEAT. RESULTS FROM EXTRACTION OF FROZEN SAMPLES INCLUDED FOR COMPARISON

VARIETY	PERIOD FOR EXOSMOSIS							
	PAD ONLY		LEAVES ONLY		TOTAL		FROZEN	
	2 HOURS	6 HOURS	2 HOURS	6 HOURS	2 HOURS	6 HOURS	2 HOURS	6 HOURS
Minhardi	288	299	198	277	486	576	1150	1830
Trumbull	490	490	562	656	952	1146	2340	3870

Table III shows that the varieties are distinguishable on a hardiness basis by extracting the paper pad only, or by extracting the residual leaf tissues. Again the results from the freezing-exosmosis method are comparable with those from the pressure method. With either treatment the tender variety yielded almost exactly double the mineral found in the extract of the hardy variety.

To test another point brought up in the discussion, namely, the question of the soluble mineral matter in the samples, the following experiment was performed. On November 16, eight 5-gm. samples of the entire tops of the plants were prepared from the varieties Minhardi and Trumbull, which were growing in field plats where they had been planted at the usual rates per acre. Of these samples, duplicates for each variety were frozen for two hours at –10° C.; others were heated, in tubes, for 20 minutes on the steam bath; two more pairs were treated with 5 cc. of ethyl ether in closed tubes for an hour; and the last duplicates were thoroughly ground with 5 gm. of washed quartz sand. To each sample, in 1×8

pyrex tubes, 50 cc. of distilled water were added and exosmosis was permitted to continue at 2° C. The results of this experiment are shown in table IV.

TABLE IV

SPECIFIC CONDUCTIVITY ($\times 10^6$) (2° C.) OF AQUEOUS EXTRACTS OF WHEAT LEAVES INJURED IN SEVERAL WAYS. EXOSMOSIS CONTINUED 20 HOURS

VARIETY	TREATMENT			
	FROZEN	STEAM BATH	ETHER	GROUND
Minhardi	639	928	695	1120
Trumbull	950	937	730	1173

These results show that while the freezing (or pressure) treatment distinguishes these two varieties very sharply, the other methods used are ineffective. The mineral contents of the extracts of heated, ground, or ether-treated samples do not differ markedly in the two varieties.

Several further experiments were performed to test the influence of the factors more commonly encountered in field samples. It was found that differences in environment, which resulted in changed plant growth, caused variations in the values obtained in these tests. Vegetative plants, grown in well worked soil, were tenderer than smaller, stocky plants grown in firm, compacted soil. Plants shaded half of each day for 8 days were tenderer than adjacent plants receiving full sunlight. When individual plants of a pure line variety were selected for the tests, considerable variation in hardiness was found from plant to plant. When ten plants of Minhardi (very hardy) and ten of Trumbull (rather tender) were so tested, they formed two distinct groups; that is, the hardiest Trumbull plant was tenderer than the tenderest Minhardi plant. In a similar test of Minhardi and Wis. Ped. 2 (both hardy varieties), the groups were by no means distinct, although the average was decidedly in favor of Minhardi, as it should be. All due precaution should be taken in growing plants for these tests, therefore, if the results are to be reliable.

Summary and conclusions

1. A method is described by means of which small samples of wheat leaves can be tested for hardiness according to the principle outlined by NEWTON. The modification of the original method consists in absorbing the expressed juice in ashless filter pads and determining the quantity of sap by measurements of electrical conductivity after extraction of the pad and the residual tissue with distilled water. Extraction of the pad, alone, gives an indication of the relative volume of sap expressed. Extraction of

the pressed tissue, itself, appears to measure the degree of crushing injury in the press. In each case, extracts of tender varieties have higher electrical conductivities than those from hardier plants. The large varietal differences in extraction after pressing are shown to correlate very well with the differences in extraction of frozen samples, according to the method of DEXTER *et al.*

2. It is demonstrated that these differences are not due to correspondingly great differences in "soluble minerals" in the samples. Extraction of plants subjected to freezing or pressure served to differentiate the varieties on a hardiness basis, while extraction of plants injured by heat, ether, or grinding did not.

3. From these facts, it would seem not improbable that a considerable part of freezing injury may be due to the mechanical injury of pressure of the ice formed (MAXIMOV **4**).

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THE OPTICAL LEVER AS A TOOL IN PHYSIOLOGICAL STUDIES

H. A. WADSWORTH

(WITH FIVE FIGURES)

Recent studies in the responses of the sugar-cane plant to varying percentages of soil moisture required the development of a technique for measuring elongation with considerable precision. A modified form of the optical lever, commonly used in physical laboratories for such measurements as the lineal expansion of metals, has been satisfactorily used in this work. So far as is known by the writer, such a device has never before been reported as a tool in plant physiology.

In its modified form the device consists of a small circular mirror mounted normally to a short brass rod which is bifurcated at the end remote from the mirror. This split end is spread to receive the plant member to be under observation. Holes drilled through the two parts of the split end, in a line parallel to the plane of the mirror, permit the pinning of the plant tissue to the end of the lever with a minimum of damage.

The weight of the lever is carried upon a metal rod carrying two projections provided with carefully machined depressions in which two steel pins attached to the mirror support are seated when the device is assembled for use. The lever is so counterpoised that its center of gravity is directly over the line of the pins in order that the plant tissue may be protected from stress when attached to the lever arm.

In use the metal rod is attached to a clamp stand at a convenient height, the mirror pins placed in the depressions, and the plant tissue pinned between the split ends of the lever arm. Vertical displacement of the point of attachment naturally deflects the mirror through an angle θ, while the reflected beam from a fixed source moves through an angle 2θ.

Observations upon the magnitude of these deflections may readily be made with a laboratory telescope provided with a cross hair and a scale mounted in the plane of the incident and reflected rays. Evidently the apparent magnification of growth depends partially upon the distance of the scale from the mirror. A distance of 1 meter has been found satisfactory with the standard laboratory telescope.

In operation the telescope is clamped horizontally at the height of the center of the mirror and focused upon the image of the scale, which is clamped to the same support as the telescope. Deflections of the mirror after setting cause the reflected beam to move down the image of the scale,

while the intersection of the cross hair gives an easily read reference point. With a lever arm of 10 cm. and a distance of 1 meter from the mirror to the scale, a change of 6 cm. per hour in the scale reading has repeatedly been noted with sugar cane. This change of 1 mm. per minute is easily observed by continuous observation.

Figure 1 shows the general assembly of two optical levers measuring the

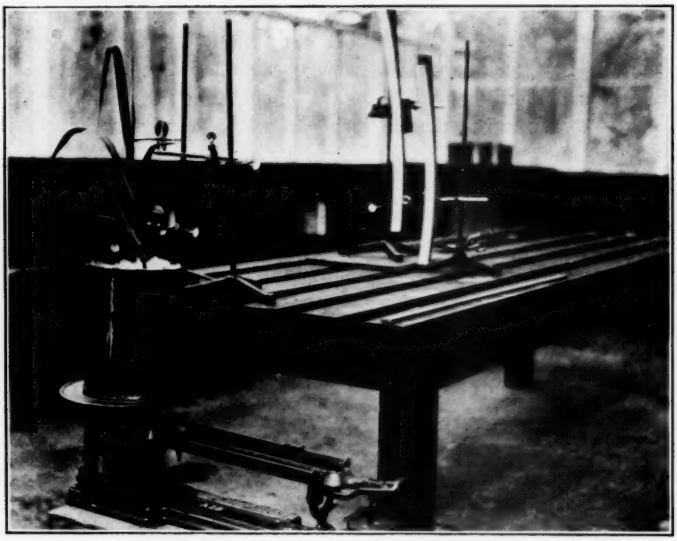

FIG. 1. Two optical levers attached to critical points on a young sugar-cane plant.

rates of growth of two critical points in a sugar-cane plant. The system is also under gravimetric control in order that some information may be had of soil-moisture conditions. A similar installation with a small pineapple seedling is shown in figure 2. In this case light stirrups of aluminum wire connect the tips of the growing leaves to the lever arms. This system as well as that in figure 1 is under gravimetric control. It should be noted that in each case the balance is securely held in a solid position by excessive weights, except when the occasional weighings are being made.

Specimen results from these two installations are shown in figures 3 and 4. In figure 3, representing observations with sugar cane, the upper curve shows the vertical displacement of a point on the leaf bundle above the growing point, while the lower curve shows the elongation of the cane itself as measured by the last visible ligule or "dewlap," as it is called locally. This structure presumably is opposite the most active meristematic tissue. The two curves in figure 4 show the growth history of two

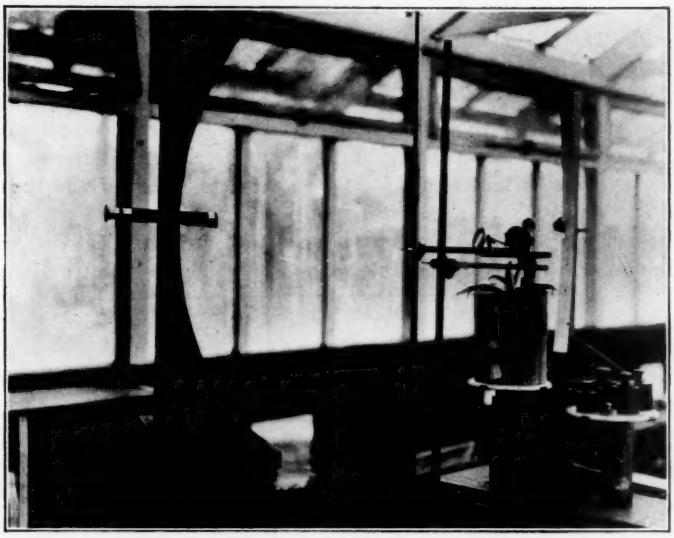

FIG. 2. Two optical levers attached to a young pineapple seedling.

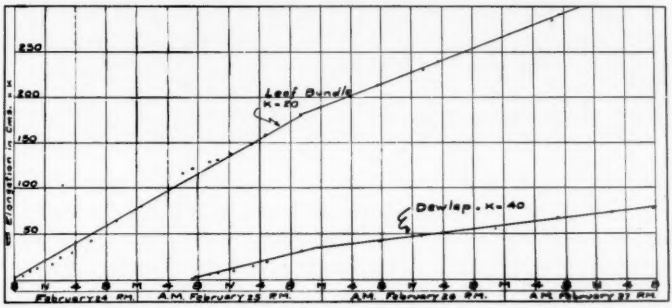

FIG. 3. Growth history with sugar cane as reported by optical levers in figure 1.

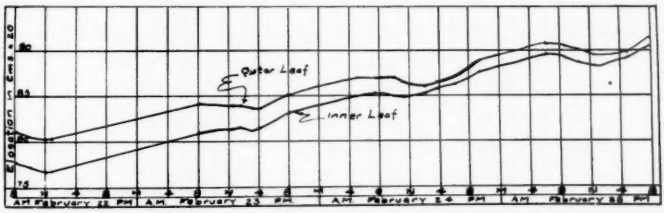

FIG. 4. Growth history of pineapple seedling as reported by optical levers in figure 2.

adjacent leaves in the inner whorl of the pineapple seedling shown in figure 2. The consistent regression during the heat of the middle of the day is interesting; but whether this phenomenon is due to the high temperature in a poorly ventilated greenhouse, to a peculiarity of the individual under observation, or to a general characteristic of the pineapple plant, is not known. The further study of this characteristic and its correlation with environmental factors making for excess rate of loss of moisture from leaf tissue is possible by means of the optical levers.

The geometry of the lever system is a little more complicated than is at first apparent. Figure 5 illustrates a case in which the point under obser-

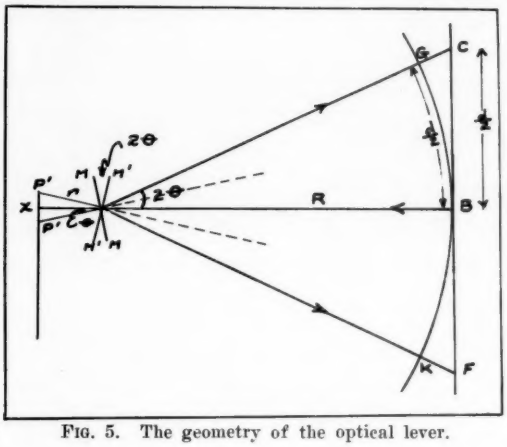

FIG. 5. The geometry of the optical lever.

vation moves from P^1 to P^1, the mirror MM and M^1M^1 swinging through the angle 2θ. The line of sight from the telescope at B bisects the angle KOG. Two scales are shown. One, CBF, is plane, normal to the line of sight and in the plane of the incident and reflected beams. The other, GBK, is in the form of the arc of a circle of radius R and in the same plane as just described.

If the straight scale be used and a direct proportion between the vertical displacement of P and the resulting difference in scale reading is desired, it is evident that the angle θ must be small in order that

$$r \sin \theta = K R \tan 2\theta \qquad (1)$$

may express the proportion within the desired degree of precision.

With the curved scale,

$$r \sin \theta = K R\ 2\theta \text{ (in radians)} \qquad (2)$$

must express the proportion with the desired precision.

In both cases K is a proportionality constant.

The paper scales normally used for telescopes of this sort are available in 50-cm. lengths. At a radius of 1 meter the subtended angles for a 50-cm. arc is about 28°. Since this angle is twice that between MM and M^1M^1, the maximum deflection of the mirror becomes 14° and the maximum value for θ is 7°.

When this value is substituted for θ in (1) and (2), the maximum error, in the case of a flat scale with a total length of 50 cm. and a gross ratio of lever arms of 1:10, is about 2.5 per cent. For the curved scale the corresponding error is about 0.2 per cent. The desirability of the curved scale is obvious, particularly with rapidly growing parts. Attaching the mirror assembly to the supporting rod by means of a clamp and slow motion screw is an obvious improvement which has not as yet been used. This would be of greatest value with such precise work that only the smallest values of θ were permissible.

It is evident, moreover, that the geometry in figure 5 demands that the growing point move vertically. This condition may be closely approached by establishing the point P at a considerable distance from any rigidly supported part of the plant.

The writer wishes to express his appreciation of the advice and assistance of Dr. WILLARD H. ELLER, of the Department of Physics, University of Hawaii, in designing the optical levers described.

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CALLUSING AND ROOTING OF COTTON STEM CUTTINGS[1]

H. E. Rea

The writer has been interested in the vegetative propagation of cotton for several years and has already made some report of his early experiments with cotton cuttings.[2,3] In the interest of developing further information on this subject, additional experiments were conducted during 1930. Early in the fall a preliminary experiment was completed on a special method of callusing cotton stems. Later a study was made of the relative adaptability of cotton and horticultural plants to propagation by cuttings, in which a large number of these pre-callused cotton cuttings were utilized.

The callusing trials were patterned after an earlier experiment conducted in 1929. In the original trial one lot of 80 cuttings was extremely successful. These cuttings were placed, inverted, in glass jars partially filled with Houston black clay which contained approximately 28 per cent. moisture. After the jars were closed loosely the "butts" of the cuttings were left protruding in the moist atmosphere above the soil surface, for six weeks. Under this condition all except one of the 80 cuttings callused profusely and, compared with the other variations included in the experiment, these cuttings were the most successful. Since the number of cuttings in the original test was small, a similar trial was made in 1930 when 1000 cuttings were used. In the second trial some of the cuttings began to callus in 10 days and in 30 days all of them were perfectly callused.

In previous studies of methods satisfactory for the rooting of cotton cuttings the writer has arbitrarily used the recommendations ordinarily made for the winter propagation of deciduous horticultural cuttings. To test the relative response of cotton cuttings to conditions which are favorable in most respects to horticultural cuttings, 1348 cotton and 1843 horticultural cuttings were placed under identical conditions in a greenhouse propagating bed. As previous experiments with cotton indicated that a relatively high temperature might be more successful, the average temperature maintained for this experiment was somewhat higher than is usual for horticultural cuttings.

The cuttings were placed in rows spaced approximately 2 inches apart and running along the short dimension of the bench. The number of cuttings per row varied inversely as their diameter, and there were usually from

[1] Contribution from the Division of Agronomy, Texas Agricultural Experiment Station, Technical Series no. 221.

[2] Rea, H. E. Asexual reproduction of cotton. Jour. Heredity **19**: 357. 1928.

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20 to 30 cotton or 30 to 45 horticultural cuttings in each row. The cotton cuttings were placed in alternate rows. Soft, medium mature, and mature wood was used in making the cotton cuttings. The cuttings were collected and prepared identically as described in an earlier paper.[3] The mature cuttings were divided into three groups for separate treatment prior to placement. One group was used without any special treatment while another lot was dipped in warm paraffin and the third lot was thoroughly callused at the time of placing the cuttings in the sand.

The horticultural cuttings used were Amur privet (*Ligustrum amurense*), lilac chaste-tree (*Vitex agnus-castus*), Japanese privet (*Ligustrum japonicum*), Vanhoutte spirea (*Spiraea vanhouttei*), Chinese arborvitae (*Thuja orientalis*), Fortune Forsythia (*Forsythia suspensa fortunei*), and pomegranate (*Punica granatum*). These cuttings were selected from the horticultural plants locally available that ordinarily root satisfactorily under a variety of conditions. All the deciduous horticultural cuttings were made from ripe wood collected just prior to frost and still retaining their leaves. The leaves were removed in making the cuttings. Chinese arborvitae was the only evergreen used and these cuttings were made in the usual manner.

Separate lots of both cotton and horticultural cuttings were set in beds of quartz and granite sand, with and without a peat substratum. After the cuttings were placed, a glass sash covering was provided for most of them. Bottom heat was supplied by means of hot water pipes under the benches and the general temperature of the greenhouse was controlled by an atmospheric thermostat set at 75° F. The temperature recorded during the experiment averaged 78° with a normal variation of 10° F.

The results secured with cotton cuttings as presented in table I show that only 8.2 per cent. of the 1348 cuttings used were successfully rooted. Over 200 cuttings each of the soft and medium mature cotton cuttings were tried without getting a single cutting to strike root. However, the three groups of mature wood cuttings rooted in excess of 10 per cent. Cotton cuttings made from mature wood immediately before using and without any special treatment gave a rooting percentage of 11.3. The pre-callusing of the cuttings was of little value as only 10.8 per cent. of that group rooted. Dipping the mature cuttings in paraffin did increase the percentage rooted to 15.3.

The results of the three variations in treatment provided for each type of cotton cutting failed to indicate any very promising method of rooting these cuttings. Heretofore the quartz sand locally available has frequently been used in attempts to root cotton. This sand invariably contains small quantities of clay and as this is generally regarded as undesirable as a cut-

TABLE I

PERCENTAGE OF COTTON AND HORTICULTURAL CUTTINGS ROOTED

TYPE OF CUTTING	NO. OF CUTTINGS USED	AVERAGE OF ALL TREATMENTS	MEDIUM USED		SUBSTRATUM		COVERING	
			QUARTZ SAND	GRANITE SAND	NONE	PEAT	NONE	GLASS SASH
Cotton stem cuttings	1348	8.2	10.0	6.5	10.1	6.6	10.1	7.3
Soft wood	235	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Medium mature wood	232	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Callused mature wood	325	10.8	16.6	4.9	13.9	7.5	11.8	10.2
Mature wood	230	11.3	6.3	14.0	15.8	6.8	12.2	10.9
Paraffin mature wood	326	15.3	19.1	11.8	15.6	15.1	20.2	12.6
Horticultural cuttings	1843	31.0	36.0	25.5	31.3	31.7	17.3	30.9
Amur privet	168	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Japanese privet	275	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vanhoutte spirea	449	26.1	31.9	19.8	22.3	29.8	16.3	30.8
Chinese arborvitae	353	39.7	41.0	38.3	41.6	37.8	19.5	49.2
Pomegranate	281	47.7	64.4	28.8	56.8	37.6	——	47.7*
Fortune Forsythia	147	51.7	62.0	39.7	44.0	59.7	——	51.7*
Lilac chaste-tree	170	61.3	62.4	59.7	60.9	61.4	63.8	58.9

* Not used in the comparison of covered and exposed cuttings.

ting medium, a pure granite sand was used[4, 5, 6]. However, only 6.5 per cent. of the cotton cuttings placed in the granite sand rooted compared with 10.0 per cent. of those in the quartz sand. Some horticultural propagators have used mixtures of peat moss as a medium with unusual success[7, 8, 9]. In the present experiment a substratum of peat moss was used under the sand in some sections of the cutting bed. This was tried in comparison with sand alone with the result that only 6.6 per cent. of the cotton cuttings with peat moss under them rooted while 10.1 per cent. of those without it rooted. Good results have been reported from some horticultural cuttings by covering the cutting bed with a glass sash[4, 5]. In this experiment a portion of the cuttings was under sash. The cotton cuttings under the sash rooted to the exent of only 7.3 compared with 10.1 per cent. for those exposed.

The results secured with horticultural cuttings are also presented in table I and show that 31.0 per cent. of the 1843 cuttings used were rooted. Although this was low for this class of cuttings it was twice as high as the best result secured from any group of cotton cuttings. Also this percentage was made in face of the fact that none of the Amur or Japanese privet cuttings rooted.

Amur and Japanese privet are both very hardy plants and usually their cuttings root even though conditions are not altogether favorable. In this experiment, however, over 100 cuttings of each of these plants were used without obtaining a single rooted cutting. An investigation of the situation indicated that the Amur and Japanese privet cuttings were killed by excessive bottom heat. In making these cuttings they were cut an inch or two longer than the others and were unintentionally placed deeper.

All of the other horticultural cuttings rooted more frequently than did any group of the cotton cuttings. Lilac chaste-tree gave the highest proportion of rooted cuttings, as 61.3 per cent. of them were successful. Also roots were produced by 51.7, 47.7, 39.7, and 26.1 per cent. of the Forsythia, pomegranate, arborvitae and spirea cuttings, respectively.

Without an exception the horticultural cuttings rooted more frequently in the quartz sand than in the granite sand. On the average, 36.0 per cent. of these cuttings rooted when placed in quartz compared with 25.5 per cent.

[4] BAILEY, L. H. The nursery book. 19th ed. Macmillan Co. pp. 44–58. 1914.

[5] HOTTES, A. C. Practical plant propagation. A. T. De La Mare Co. pp. 61–85. 1917.

[6] YERKES, GUY E. Propagation of trees and shrubs. U. S. D. A. Farmers' Bull. 1567. 1929.

[7] ESPER, H. C. The effect of time of taking, medium, and bottom heat on the rooting of evergreen cuttings. Ohio Agr. Exp. Sta. Bimonthly Bull. 154: 9–17. 1932.

[8] HITCHCOCK, A. E. Investigations in propagation. So. Florist & Nurseryman 27: no. 13. 12, 27. 1929.

[9] REUDEL, FRANKLIN J. Methods of propagation. So. Florist & Nurseryman 25: no. 17. 8–9. 1928.

in granite sand. The proportion of horticultural cuttings rooting with and without a peat moss substratum were 31.7 and 31.3 per cent. respectively. Although there was little difference in the composite results, the use of peat moss appeared to have increased the percentage of rooted cuttings for spirea and Forsythia. On the other hand, the pomegranate cuttings were injured by peat moss while chaste-tree and arborvitae cuttings rooted just as readily whether peat moss was used or not. Undoubtedly the horticultural cuttings were favored by the use of the glass sash. Since none of the pomegranate or Forsythia cuttings was exposed, their records were eliminated from this comparison. The average results of the other horticultural cuttings show that 30.9 and 17.3 per cent. of the covered and exposed cuttings rooted, respectively. However, the chaste-tree cuttings rooted satisfactorily whether covered or exposed.

The main results are briefly summarized.

1. The results obtained with cotton cuttings confirm the results on callusing obtained previously.

2. Under identical conditions, the proportion of cotton cuttings striking root in these experiments was markedly lower than for lilac chaste-tree, Fortune Forsythia, pomegranate, Chinese arborvitae, or Vanhoutte spirea.

3. The selection of mature wood is important in the winter propagation of cotton cuttings. The soft and medium mature cotton cuttings used in his experiment were worthless.

4. The pre-callusing of mature cotton cuttings failed to prove of value but dipping the cuttings in paraffin increased the percentage of rooting slightly.

5. The highest percentages of rooting with mature cotton cuttings were secured in an exposed greenhouse bed of quartz sand free of any substratum.

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BRIEF PAPERS

RESPONSE OF IRIS TO SOIL REACTION

(WITH TWO FIGURES)

Recommendations in popular literature vary considerably as to the optimum soil reaction for the Bearded iris, *Iris germanica*, ranging from "acid" to "alkaline." DYKES[1] states that "The Bearded Irises all, without exception, want dry conditions and the only hope in damp situations is to make raised beds for them. They also need lime in the soil and this is, in most cases, best supplied in the form of old mortar rubble. The Apogons do not, as a rule, want lime, except possibly the Spurias and some, such as the Californians, will not grow in a soil that is highly charged with lime."

Since soils on which the iris is grown in Ohio vary widely in reaction, it seemed desirable to determine the optimum condition or range for them. A series of plots on a raised bed in the greenhouse, which had already been prepared for studying other species of flowers, was used for the purpose.[2] These plots were 7×8 feet in size and were filled with Canfield silt loam, in June, 1929. The pH value of the soil was 5.0 at the time it was moved into the greenhouse. A one-inch layer of German peat (pH 3.5) was added in an endeavor to improve the humus content. Increments of aluminum sulphate or hydrated lime were added to the plots until a range of pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 was attained for the eight plots respectively. Readings of the pH value of each plot were made biweekly, either electrometrically or by means of the La Motte soil tester.

On September 1, 1930, irises were planted in these plots, using Alcazar and another German variety, the name of which was not determined. The rhizomes were approximately the same size at the time of planting, but the weights were not recorded. Three plants of each variety were set to the plot and they received identical care with the exception of occasional additions of aluminum sulphate or hydrated lime as was necessary to maintain the desired reaction of the soil.

For the first four months no appreciable differences were noted in the growth or color of the plants. Beginning January 1, 1931, a darker color and larger size of foliage were noted in the plants growing on plots 7 and 8 (pH 7.5 and 8.0 respectively). Plots 1 and 2 by this time showed a somewhat stunted appearance, with some tip burn. The succeeding plots were

[1] DYKES, W. R. Handbook of garden irises. Hopkinson. 1924. p. 235.

[2] WIGGIN, W. W., and GOURLEY, J. H. Studies on the reaction of greenhouse soils to the growth of plants. Ohio Agr. Exp. Sta. Bull. 484. 1931.

increasingly better, particularly in the alkaline ones, as noted. Throughout the following eighteen months there was a gradual widening of the growth behavior between plots 1 and 8 until those in the former were practically worthless. Whether this injury to the plants at pH 4.5 and 5.0 was due to the high concentration of hydrogen ions or to toxicity due to soluble aluminum was not definitely determined, but probably the latter played an important rôle.

Figure 1 shows a typical sample of leaf from each plot. There is a

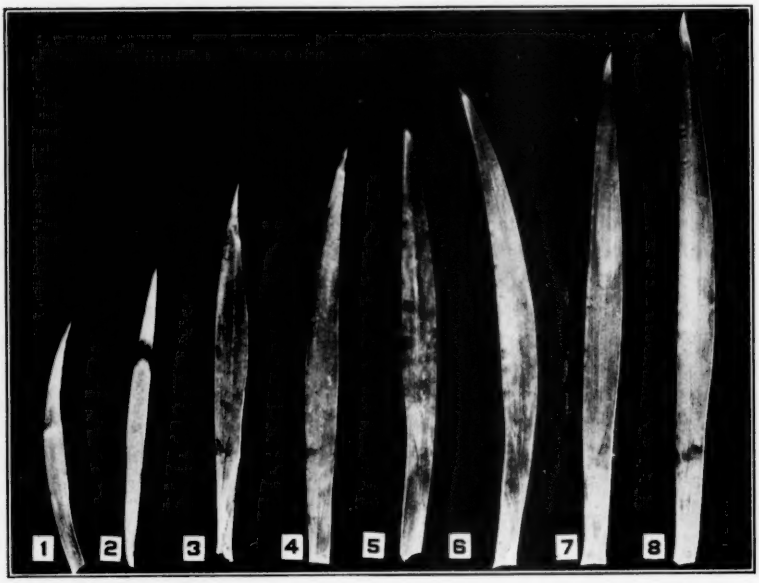

FIG. 1. A typical leaf from soil reaction plots having a range of pH 4.5 to 8.0.

fairly consistent gradient in size, color and amount of tip burn from plot 1 to plot 8.

Figure 2 shows a typical plant from each plot, photographed June 15, 1931.

At the time of removal from the bed, July 22, 1932, the Alcazar plants were weighed and the length of typical leaves measured. With one slight exception, plot 5, the weight is in an ascending order from plot 1 to 8, increasingly higher as acidity decreases; that is, from plot 1 to 8. The length of flower stems was measured on June 15, 1931, and, with other data, are shown in table I.

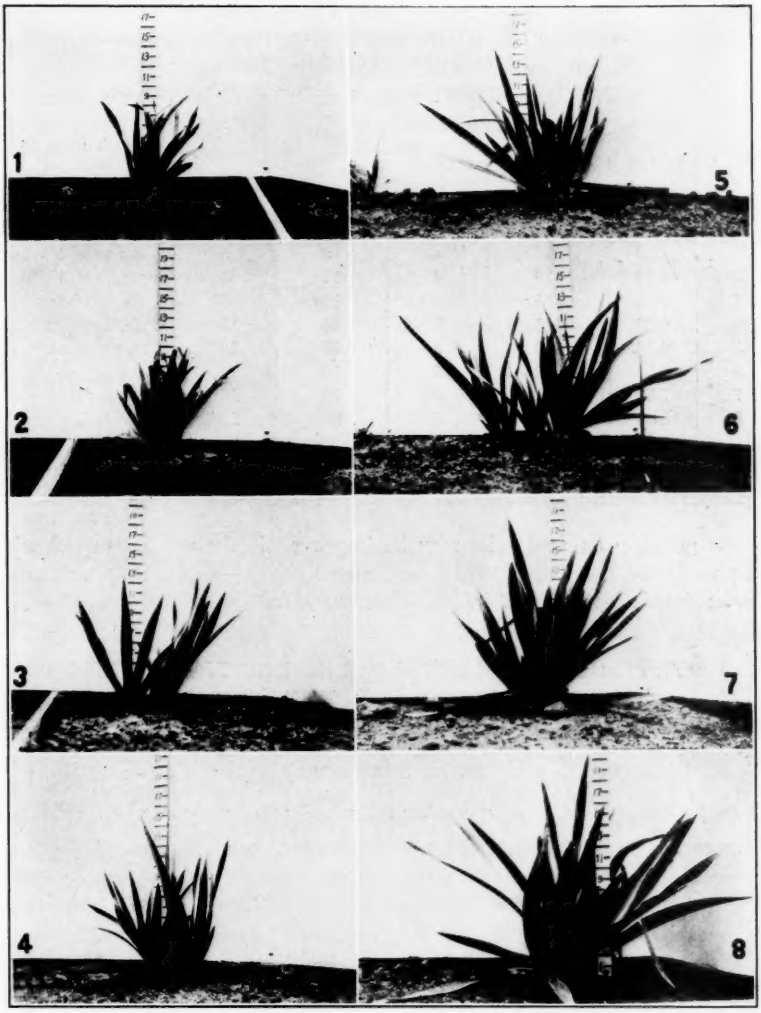

FIG. 2. Alcazar iris plants typical of those grown in soil reaction plots from pH 4.5 to 8.0.

From this work it appears that the German iris, Alcazar, thrives much better when the soil reaction is nearly neutral to alkaline than in acid soils. Since these plants were kept rather dry throughout to prevent injury, the matter of wet soil is eliminated. The conclusion may be drawn that iris

TABLE I

SOME EFFECTS OF SOIL REACTION ON GROWTH OF ALCAZAR IRIS PLANTS, SEPT. 1, 1930, TO JULY 22, 1932

PLOT	RE-ACTION	AVERAGE WEIGHT OF 3 PLANTS	AVERAGE NUMBER OF SHOOTS PER PLANT	AVERAGE HEIGHT OF FOLIAGE	LENGTH OF FLOWER STEMS, JUNE 15, 1931	CONDITION OF FOLIAGE
	*pH**	*gm.*		*inches*	*inches*	
1	4.49	276	4	9	3	Very pale, tip burn
2	4.86	316	4	11	5	Very pale, tip burn
3	5.46	385	6	13	8	Pale, tip burn
4	6.05	401	5	14	10	Pale, tip burn
5	6.48	393	5	15	10	Green
6	6.93	529	5	16	14	Dark green
7	7.56	561	4	18	16	Dark green
8	8.05	677	5	20	22	Very dark green

* The last reading before the iris plants were removed, recorded electrometrically with a quinhydrone electrode, June 21, 1932.

plants growing on acid soils may be improved if the soil is treated with lime until the reaction is nearly neutral or above.—J. H. GOURLEY, *Ohio Agricultural Experiment Station, Wooster, Ohio.*

GROWTH OF TREE SEEDLINGS IN RELATION TO LIGHT INTENSITY AND CONCENTRATION OF NUTRIENT SOLUTION[1]

(WITH ONE FIGURE)

The importance of available mineral nutrients in the shade tolerance of trees has been emphasized by LUNDEGARDH,[2] who points out that the presence of abundant mineral nutrients in the soil favors chlorophyll and foliage formation and thus increases the assimilative capacity of plants under shade conditions. Sufficient experimental data relative to the effects of mineral nutrients on plants grown at low light intensities are lacking, however. The following experiments were carried out to determine the effects of concentration of mineral nutrients on tree seedlings grown at or near the minimum light intensity required for growth.

[1] The author wishes to express his appreciation to Dr. R. B. HARVEY of the University of Minnesota for his encouragement and suggestions during the course of the experimental work and for the material assistance and facilities offered by the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station.

[2] LUNDEGARDH, H. Klima und Boden in ihrer. Wirkung auf das Pflanzenleben. Jena. 1925.

Seedlings of *Fraxinus pennsylvanica* were germinated in quartz sand and then transferred to nutrient solutions of the same composition but varying total concentrations. The solution used was a three-salt solution, consisting of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, corresponding in molecular proportions to solution R4S1 of the National Research Council Bulletin on "A plan for cooperative research on the salt requirements of representative agricultural plants."[3] Iron was added in the form of ferric phosphate in the concentration of approximately 3 parts per million. Three concentrations of solution were used corresponding to 1.0, 0.1, and 0.01 atmospheres, and the solutions were renewed at four-day intervals during the growing period.

The seedlings were grown in four light intensities, obtained by use of electric lights and corresponding to 130, 70, 48, and 31 foot-candles as measured with the Macbeth illuminometer. Previous experimentation had indicated that the minimum light intensity for growth was approximately 50 foot-candles when using continuous illumination. Temperature was kept at 70° F. with a maximum variation of about 2 degrees. Relative humidity was maintained at approximately 80 per cent.

At the end of a ten-week growing period the seedlings were removed from the jars and compared as to gain in dry weight, length of roots and

TABLE I

COMPARISON OF THE GROWTH AND DEVELOPMENT OF *Fraxinus* SEEDLINGS IN THREE LIGHT INTENSITIES AND THREE CONCENTRATIONS OF NUTRIENT SOLUTION

LIGHT INTENSITY	SOLUTION CONCENTRATION	GAIN IN DRY WEIGHT*	LENGTH†		WATER TRANSPIRED PER GM. DRY WEIGHT GAINED
			TOPS	ROOTS	
foot candles	*atm.*	*gm.*	*mm.*	*mm.*	*gm.*
130	1.00	3.20	65	218	370.4
130	0.10	3.27	72	150	439.4
130	0.01	2.43	61	131	374.5
70	1.00	1.70	39	175	
70	0.10	1.74	51	119	498.1
70	0.01	1.50	49	117	
48	1.00	0.84	53	120	583.9
48	0.10	0.86	58	107	971.6
48	0.01	0.77	51	100	843.2

* Average of 24 individuals.
† Average of 39 individuals.

[3] LIVINGSTON, B. E., CROCKER, WM., and KELLERMAN, K. F. A plan for cooperative research on the salt requirements of representative agricultural plants. Baltimore. 1919.

stems, general vigor, and the amount of water lost in transpiration. The results are summarized in table I and figure 1 and refer to the seedlings grown in the three higher light intensities since the plants grown in the lowest light intensity did not survive more than one or two weeks in any of the nutrient solutions.

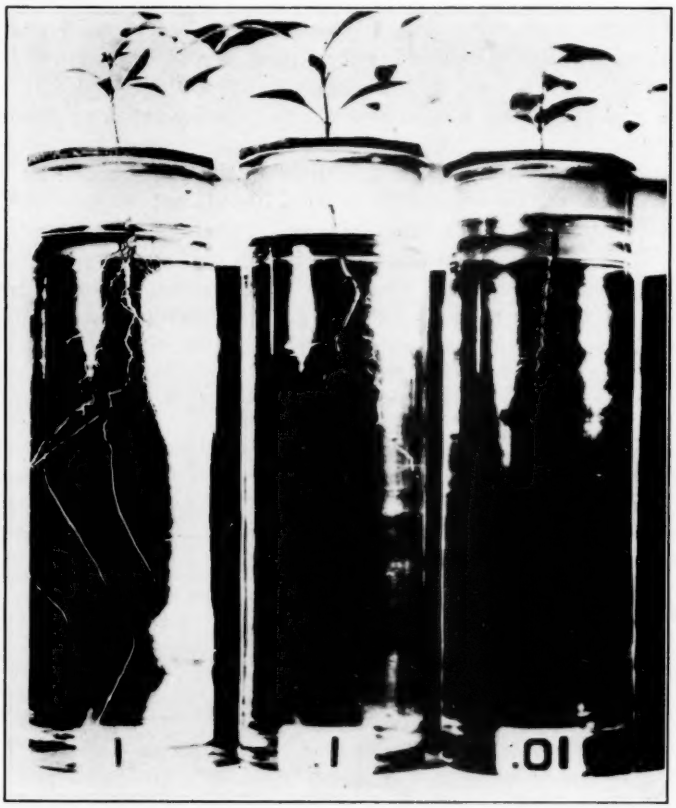

FIG. 1. Photograph showing effect of concentration of nutrient solution on type of root growth.

The data obtained, relative to the effect of concentration of nutrients on light requirements of the tree seedlings used, indicate that while a sufficient supply of nutrients must be present to satisfy the needs of the plant, the minimum light requirement cannot be lowered by increasing the amount of available nutrients. The length of the period of survival for the

seedlings grown under a sub-minimum light intensity was the same in all cases regardless of the solution concentration.

The greatest response to an increase in concentration of the nutrient solution occurred at the higher light intensities, indicating a greater need for nutrients under those conditions. Thus increasing the concentration of solution from 0.01 to 0.1 atm. for a light intensity of 48 foot-candles resulted in an increase in dry weight of 11.7 per cent. For seedlings growing under light intensities of 70 and 130 foot-candles there resulted increases of 16.0 per cent. and 34.5 per cent. respectively.

The marked increase in total length of roots developed in a solution of 1.0 atm. concentration over those grown in a solution of 0.1 atm. was more than offset by a greater abundance of lateral branching at the lower concentration. The effect of concentration on type of root growth is illustrated in figure 1.

The water requirement, expressed in terms of grams of water transpired per gram of dry material gained, might seem to indicate a greater need for water at the lower light intensities. This, however, was due to a reduction in the rate of assimilation of material rather than to a high intake of moisture.—GEORGE P. STEINBAUER, *Minnesota Agricultural Experiment Station, St. Paul, Minnesota.*

METHODS OF MEASURING STRAWBERRY LEAF AREAS

In MILLER'S[1] recent text on plant physiology, five methods of measuring the leaf surface of plants are given. In the writer's work with strawberries during the past year, however, three other methods were used, none of which was mentioned in this text. As these methods were adopted as more useful under the conditions encountered for field and greenhouse work than those given by MILLER, it seems worth while to call attention to them. The first and second are not new but apparently are not as well known as they should be. The third is apparently little known. They are (1) the leaf-product, (2) the matching, and (3) the adjustable platform-planimeter methods.

LEAF-PRODUCT METHOD.—This method, used by LIVINGSTON and associates,[2] is based on the correlation between extent of leaf surface and lineal measurements of the leaves. It provides an index of the actual area of varying degrees of accuracy. The writer[3] has used the following

[1] MILLER, E. C. Plant Physiology. McGraw-Hill Co. 1931.

[2] MCLEAN, F. T. A preliminary study of climatic conditions in Maryland, as related to plant growth. Physiol. Res. **2**: 129–208. 1917.

[3] DARROW, GEO. M. Experimental studies in the growth and development of strawberry plants. Jour. Agr. Res. **41**: 307–325. 1930.

indices in actual practice according to the degree of accuracy required: length of middle leaflet; length × breadth (leaf product) of middle leaflet; and length × breadth of all three leaflets of the strawberry leaf. These indices were accurate to within 5 per cent., the single measurement of length being least accurate; the leaf-product (length × breadth) of the middle leaflet more accurate; and the sum of the leaf-products of all three leaflets the most accurate index of actual area. The accuracy of this index is obviously dependent on (1) the number of measurements used to obtain a factor to translate leaf length or length × breadth into area; (2) whether the measurements obtained are for one or for several varieties; and (3) the uniformity of the conditions under which the plant material is grown. Under this method may be listed that of CARUTHERS,[4] in which breadth was used as an index of leaf area of *Ribes* much within an accuracy of 5 per cent.

MATCHING METHOD.—This method has been used by several investigators, but was described to the writer by C. F. KINMAN, of the Division of Horticultural Crops and Diseases, who used it to obtain the area of great numbers of leaves of pears and other fruits. For this a pasteboard form equal to the largest leaf encountered is cut out, its area measured with the planimeter and the size written on the form. On this form are drawn outlines of successively smaller leaves, each of which is measured and the area recorded on the form corresponding to each. Leaves on trees or in the laboratory are matched rapidly and with an accuracy (table I) approaching or equal to that when a planimeter is used. The more uniformly shaped leaves are measured with the greatest accuracy and rapidity (several hundreds per hour) by this method. Even actual areas of leaves of many plants can be obtained almost as quickly as length alone can be measured. The matching of strawberry leaves, however, is difficult as compared with matching of leaves of some other plants; yet it has been possible to follow the increase and decrease in leaf area of hundreds of experimental plants in the field and greenhouse with great accuracy and with relative ease. The use of several pasteboard forms of different sizes fastened to heavy cardboard strips (8 small, 6 medium, and 4 large in our work) was found to be more than 20 per cent. faster than a single form with concentric outlines of smaller leaves on it. If very accurate data are wanted, forms for each variety must be used; otherwise a set of forms for all varieties can be used.

ADJUSTABLE PLATFORM-PLANIMETER METHOD.—This method was suggested by W. M. ATWOOD, of the Oregon State College. To one edge of an adjustable stand used for camera lucida drawings a piece of automobile

[4] CARUTHERS, R. S. A scale for measuring areas of *Ribes* leaves. Phytopath. **19**: 399–405. 1929.

celophane is so fastened by means of thumb tacks that a leaf can be inserted under the celophane but on the stand. The stand, quickly adjustable to any angle in any direction, is used to hold the leaf and the planimeter for measurements of actual areas. Leaves on shrubs and herbs within easy reach of the ground can quickly be measured. Such a stand was found especially useful when measuring leaves of experimental strawberry plants in the greenhouse.

RELATIVE SPEED AND ACCURACY.—The relative speed and accuracy of the three methods and of variations of each were calculated for one variety, O. S. C. 192 (Marshall × *Fragaria chiloensis*). In a first test of speed and accuracy, 10 leaves were used to obtain correction factors and these applied to measurements of 18 leaves (54 leaflets). In the second test 50 leaves were used to obtain correction factors and these applied to measurements of 50 other leaves. Results of both tests are given in table I.

TABLE I

COMPARISON OF SPEED AND ACCURACY OF METHODS FOR OBTAINING LEAF AREAS OF STRAWBERRIES

	PLANIMETER	MATCHING	LENGTH	LENGTH × BREADTH
No. leaves per hour				
First test	48	156	136	114
Second test	21	172	172	108
Percentage of speed				
First test	31	100	87	73
Second test	21	100	100	63
Percentage of error				
First test	0.0	3.2	4.3	4.7
Second test	0.0	1.8	4.3	0.2

As to speed, the results were surprising even to those using the methods. The matching method was, on the whole, faster than that of measuring length only, and was considerably more accurate. When 50 leaves were measured as in the second test, the accuracy of the length × breadth method was 0.2 per cent. and of the matching method 1.8 per cent. It is probable that under ordinary conditions either the matching or length × breadth methods are as accurate as using the planimeter itself. It should be remembered that in these tests the use of 50 strawberry leaves meant measuring 150 leaflets on the plants, and that with plants having simple leaves the number measured per hour should be at least three times as great as with the strawberry.—GEORGE M. DARROW, *U. S. Department of Agriculture, Washington, D. C.*

NOTES

Atlantic City Meeting.—The ninth annual meeting of the American Society of Plant Physiologists will be held at Atlantic City, New Jersey, during convocation week, December 27–31, 1932. The headquarters for the botanical societies is the Traymore Hotel. Announcements regarding railway fares, hotel rates, etc., will be published in Science. Close to the great eastern centers of population, Atlantic City offers opportunity for a large gathering. The meeting will be notable for the SACHS centennial celebration, and for the third award of the STEPHEN HALES prize. Members of the Society are urged to include the annual dinner in their plans, as the life membership award and the STEPHEN HALES award are both usually announced at the annual dinner. In the past the banquet has been one of the most pleasant features of the entire meeting.

Summer Meeting.—The recent summer meeting at the University of Wisconsin, which was held jointly with the corn belt section of the American Society of Agronomy, July 11–13, 1932, was greatly enjoyed by those who were able to attend the various sessions and share in the profitable laboratory and field demonstrations. The attendance of plant physiologists was estimated roughly at about 40, and the total attendance at about 180. Of special interest to the physiologists was the exhibition of portraits of plant physiologists which have been collected during recent years by Dr. R. B. HARVEY of Minnesota. In connection with the exhibition HARVEY gave an illustrated lecture on his experiences among the Russian people several years ago. Much appreciated, also, was the interesting discussion of the "Relations between plant composition and growth responses" by Dr. H. R. KRAYBILL of Purdue University.

The laboratory demonstrations of experimental work on hardiness, photosynthesis, nitrogen fixation, soil fertility testing, corn seed drying, etc., were very attractive. The field trips were used to demonstrate such matters as disease control measures, hybridization and selection of various crops, and cutting practices with reference to the permanence of alfalfa stands.

The boat trip on Lake Mendota was a delightful experience for those who were able to take it. The meetings were decidedly effective, and everyone enjoyed the splendid fellowship and cordiality of the occasion.

Syracuse Meeting, A. A. A. S.—The Syracuse meeting of the A. A. A. S. and affiliated societies was small, but made up in genuine enjoyment what it lacked in numbers. Indeed, mere size has become a detriment to good

meetings. Few papers were read, a decided improvement over the winter arrangements. The main botanical addresses were by Dr. R. H. TRUE and Dr. W. L. BRAY, and there was a symposium on cell wall chemistry. The main entertainment was in the form of field trips, and these were planned and handled in excellent fashion. No one who went on the delightful excursion to the plunge-basin lakes of the Jamesville region, and to the home of *Scolopendrium vulgare*, will ever forget it; nor the all-day trip to the marl and peat bogs of West Junius; nor the afternoon at the salt flats near Lake Onondaga. If ever in doubt as to the most appropriate thing for a summer meeting, plan an interesting field trip. Nature yields a symposium suited to all tastes, and she never talks too long!

Julius von Sachs.—The hundredth anniversary of the birth of JULIUS VON SACHS is at hand. Born October 2, 1832, we are happy to honor his memory in 1932. During the year PLANT PHYSIOLOGY has carried portraits of the peerless organizer of this field of Botany, and photographs of his famous laboratory at Würzburg. It is with great pleasure that we present with this issue an unpublished photograph of SACHS at the age of about 40 years (1872), with several of his students. Seated at the right of SACHS is HUGO DE VRIES. Standing behind him is JOSEF SCHUCH, and at the right, standing, is J. BARANETZKY. It is an exceedingly interesting group, and emphasizes the inspirational leadership of SACHS during that period of botanical development. Botanists, and particularly plant physiologists, are greatly indebted to SACHS, whose ability as an investigator and teacher places him in the front rank of the late 19th century scientific leaders. May the plant physiologists of this country follow worthily but humbly in his footsteps, to build an appropriate superstructure upon the immortal foundations which he laid for us.

Incorporation.—Without the slightest disturbance to indicate the transpiring of important events, the old American Society of Plant Physiologists went out of existence, and a new society was born. The incorporators of the American Society of Plant Physiologists met at 4:20 P. M. on July 5, 1932, in room 342, West Wing of the U. S. Department of Agriculture Building, Washington, D. C. Those who met were Dr. P. F. CULLINAN, Dr. ANNIE M. HURD-KARRER, Dr. CHARLES F. SWINGLE, Dr. B. C. BRUNSTETTER, and Dr. JOHN W. ROBERTS. Dr. CULLINAN was elected temporary chairman, and Dr. ROBERTS, temporary secretary.

"It was moved, seconded, and passed by unanimous vote, that all the present existing members of the unincorporated society be elected members of the incorporated society, with their present rights and privileges."

"It was moved, seconded, and passed by unanimous vote, that the meeting adjourn till the next regular meeting of the American Society of Plant Physiologists." (Signed) JOHN W. ROBERTS, temporary secretary.

With these simple rites the Society begins its corporate existence. The way is open now for larger opportunities and larger responsibilities. A solid foundation has been laid, upon which the future development can securely rest.

Treasurer's Report.—During the last fiscal year the financial strength of the Society was put to its severest test so far. It became necessary, because of the earlier issuance of the quarterly numbers, to pay for five issues in a single fiscal year. The additional cost was about $1000. In spite of this extraordinary expense, the assets of the Society on July 1 were as great as they were a year ago, which means that even in a year of severe depression we have been able to meet this strain without resorting to any financial expedients. This fact should be a source of great satisfaction to all officers and friends of the Society. In addition to this cheering situation, the BARNES Life Membership fund has reached a total of $2105, within $400 of the amount required to finance this fund permanently. The STEPHEN HALES prize endowment is $1100, and there is $164 in the general endowment fund. There are, besides these funds, reserves and moneys on deposit that bring the total assets in cash and bonds to over $8000. At the same time, unsold volumes of PLANT PHYSIOLOGY are held to a value of over $2000. This report shows marvelous progress for a struggling young Society that started publication in 1926 with nothing but faith in the worthwhileness of the tasks which were being undertaken. With the extraordinary strain of the finances of 1931–32 behind us, and with watchful care of the expenditures for publication, it is hoped that the next treasurer's report may indicate a resumption of financial growth.

Plant Physiology.—During the last year PLANT PHYSIOLOGY has just about held its own in number of subscribers and memberships in the Society. Under ordinary circumstances there would have been a handsome gain in support. But the losses of membership were much larger than normal, so that the secretary's fine work merely succeeded in holding our ground. In order that we may continue publication at the present rate, we are appealing to our members to maintain support of the journal during the depression. If each member will do what he can to find new support among those who would find membership in the Society valuable, enough will be found to offset those whose financial condition forces them to drop membership temporarily. Extension of the library support wherever pos-

sible would also be very helpful. As several years of back volumes are available, new subscribers should be encouraged to purchase all of the back numbers which are now obtainable. Such sales increase the permanent cash assets, and by interest income from these, provide enlarged funds for the Society's work.

Stephen Hales Prize Committee.—In accordance with the provisions of the by-laws governing the STEPHEN HALES prize award, the committee in charge of the award consists of Dr. A. L. BAKKE, Iowa State College, chairman; Dr. D. R. HOAGLAND, University of California; and Dr. W. W. GARNER, U. S. Department of Agriculture.

Life Membership Committee.—President D. R. HOAGLAND has appointed on the CHARLES REID BARNES Life Membership Committee the following members: DR. W. F. LOEHWING, University of Iowa, chairman; DR. C. DEUBER, Yale University; DR. R. P. HIBBARD, Michigan State College; DR. E. S. JOHNSTON, Smithsonian Institution; and DR. R. E. GIRTON, Purdue University.

Errata.—Attention is called again to the errata list at the close of the table of contents. Insertion of these corrections in the text of your volume is recommended. The assistance of authors in compiling the list is acknowledged with thanks.

Environmental Factors in Relation to Plant Disease and Injury.—A very valuable bibliography of this field has been prepared by Dr. J. D. WILSON, and published as Technical Series Bulletin no. 9, of the Ohio Agricultural Experiment Station. The price is $1.00 per copy. It contains well toward 400 references, and is indexed in such a manner as to be exceptionally usable. There is an Environmental Factor index, following which these factors are listed in alphabetic order, with the citations (serial numbers) which consider them. The main bibliography itself is alphabetically arranged by authors. A host-disease-injury index, and a host index with scientific names in alphabetic order closes the work. The bulletin carries over 200 pages of indexed material. For sheer helpfulness it would be hard to imagine anything more useful in a bibliography. Everyone interested in environmental factors in health and disease of plants will find the bulletin indispensable to thoroughgoing work in this field. The author and the station may justly take pride in this unique contribution to the facilities for research in a very complicated field of plant physiology and pathology.

Leaf Roll Disease.—One of the Monographien zum Pflanzenschutz, no. 8, deals with the leaf roll disease of the potato. It is by Dr. F. ESMARCH, and is published by Julius Springer, Linkstrasse 23–24, Berlin W9. The monograph approaches the problem from the historical and geographic distribution standpoint, and the growing significance of the disease in agriculture. It takes up the histology and physiology of the disease, especially the effects upon carbohydrate and protein metabolism, respiration and transpiration. The methods of transfer are discussed, the influence of environmental factors upon it, the internal factors, and the etiology of the disease. The price in brochure binding is 9.9 RM. Orders may be sent directly to the publishers.

Anthokinetics.—An attractive and interesting bulletin on the physiology and ecology of floral movements has been published recently by the Carnegie Institution, of Washington, D. C., as Bulletin no. 420. The authors are Dr. G. W. GOLDSMITH and Dr. A. L. HAFENRICHTER.

The first chapter presents a resumé of the previous work on floral kinetics, the present state of knowledge concerning the mechanism of movements of floral organs, and the methods used in the observational and experimental work. To provide controllable conditions of temperature, humidity, and light, a chamber called an ecostat was developed. This control chamber is described in detail. The observations of flower movements were made under accurately controlled conditions. The species studied fall into four habit groups: heteranthous, ephemeral-heteranthous, nyctanthous, and ephemeral-nyctanthous.

Following the introduction there are eight sections dealing with particular species, which include *Tulipa gesneriana, Taraxacum officinale, Aster bigelovi, Tragopogon porrifolius, Eschscholtzia californica, Gentiana amarella, Mentzelia nuda,* and *Oenothera caespitosa,* with many others of similar habit.

The final chapter discusses the field behavior, the behavior under controlled conditions, temperature relations, light relations, humidity effects, and the mechanism of floral movements as exhibited by bracts, sepals, and petals. The work is profusely illustrated, with 34 text figures and 29 plates, 27 of which are of beautiful photographs arranged to show the cycle of floral movements of as many different species during their blooming period. These alone make the bulletin very attractive. Copies may be obtained from the Carnegie Institution of Washington, D. C., at $3.00 per copy in paper binding, and at $4.00 in cloth.

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